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respiratory chamber, with that of the standard colour solution of known hydrogen ion concentration in the test tube, the diameter of which being approximately equal to that of the respiratory chamber.

Sometimes the gill piece was mounted on a hollow glass, or was supported on a piece of thread extended just beneath the cover slip of the respiratory chamber described above, in order to bring the gill tissue near the objective lens of the microscope, when high magnification was required.

The excised gill tissue of *Pecten* survives and continues ciliary movement for many days in good condition, if kept properly in the open glass vessel with sea-water. It was also ascertained at first that the pH indicators were not perceivably toxic as far as the present experiment was concerned. In the experiments, the preparation of the gill tissue was allowed to respire in the closed respiratory chamber, and the ciliary movement continued for a considerably long period, if the medium was ordinary sea-water. As the time elapsed, however, the movement of the cilia gradually slackened and was brought to rest at last. Whether the stoppage of cilia is due to the lack of oxygen or to the effect of carbon dioxide produced by the tissue respiration was made out in the experiment given below.

The time required for the cessation of the ciliary movement in the normal sea-water in the closed respiratory chamber varies according to the size of the piece of the tissue and of the chamber. The ciliary movement keeps the medium in circulation and in a sufficiently uniform condition. We often observe, however, that the cilia do not stop at the same time in all parts of the gill piece. This is certainly due to the differences of the oxygen tension caused by local obstruction of circulation in such a small confined space of the chamber. This disadvantage could practically be overcome by agitating the medium, inverting the vessel from time to time. The gill piece which sinks to the bottom by its own weight when the vessel is inverted stirrs up the medium.

When the sea-water was acidified and the hydrogen ion concentration was sufficiently high, the ciliary movement was stopped immediately. This effect was quite distinct and could easily be observed.

For the determination of the hydrogen ion concentrations of the medium, the Clark and Lubs' series of indicators were used. As the complete data for correction of salt errors with sea-water are not on hand, the values given in this paper are uncorrected, unless otherwise stated. The salt errors of the indicators employed are, however, of the order of magnitude of 0.15 to 0.35 pH unit with 1/2 M NaCl, but there exist some

discrepancies among the data obtained by different authors[1].

The salt errors of the indicators are, however, fortunately in the same direction, and the magnitude of errors is not very different. For the comparison of the effect of pH values caused by different acids, our determinations will suffice practically. Subtraction of 0.25 on average from the observed values given in this paper may give more approximate ones. The correct pH value of the normal sea-water of the laboratory was electrometrically determined and found to be pH 8.2. (KOKUBO, p. c.).

EXPERIMENT.

Effect of lack of oxygen and effect of CO_2.

The piece of gill tissue was enclosed in the respiratory chamber with sea-water and was allowed to continue ciliary movement. Table I summarises the observations upon the cilia. In this example, it required two hours to bring the cilia to complete cessation of movement, when the hydrogen ion concentration in the medium fell down to pH 8.15 owing to the accumulation of carbon dioxide produced by the respiration of the gill tissue. Two and half hours later, the respiratory chamber was opened and the medium was renewed with sea-water acidified with CO_2 down to pH 7.0, and the vessel was again closed. Five minutes after the renewal, the cilia showed recovery and soon resumed active movement. One and a half hours later, the pH of the medium decreased below 6.8 and the ciliary movement came to rest. The medium was renewed again, therefore, with sea water acidified with CO_2 to pH 6.7, then the cilia resumed the movement promptly. The movement continued for two and a half hours, when the pH of the medium had fallen to 6.6. The preparation was allowed, after the cessation of the movement, to stand over night in the respiratory chamber, and the pH value of the medium was found to be 6.5 at the twenty fourth hour of experiment, in the next morning. The cover slip was removed after the observation and the medium was exposed to the air. After one hour, the pH value of the sea-water in the vessel rose to 7.1 as the CO_2 had escaped, and cillia decidedly recovered and began to beat, though not very actively at first.

From the foregoing experiment it may be inferred that the ciliary movement of the gill piece continues for about two hours in the closed vessel, and the movement could recover even when the medium was renewed

[1] CLARK 1928; KOPACZEWSKI 1926; HARVEY 1928; KOKUBO, *p. c.*

TABLE I.

Experiment No. 2. Effect of lack of oxygen.
Temperature: 20.5-23.0°C. Size of gill tissue: 16 mm. × 6 mm.

Date	Time of experiment		pH of medium*	Ciliary movement
	hr.	*min.*		
Sept. 12, 9:00 a. m.		0	8.2	Active
		30	8.2	do.
	1	0	8.2	A little slackened.
	1	30	8.15	Slackened.
	2	0	8.15	Completely ceased.
	4	0	8.10	do.
	4	30	—	Medium renewed with sea-water carbonated to pH 7.0
			7.0	
	4	35	7.0	Cilia began to beat
	5	0	6.8	Active
	5	30	6.8	Slackened
	6	0	6.8	Stopped
	6	10	—	Medium changed with carbonated sea-water, pH 6.7
			6.7	
	6	30	6.7	Active
	7	30	6.6	Inactive
	8	30	6.6	do.
	9	0	6.6	Stopped
Sept. 13, 9:30 a. m.	24	0	6.5	Stopped. Cover glass removed after observation
	25	0	7.1	Movement recovered

*pH values higher than 6.8 are corrected for salt error, which is about 0.2 for P R. and T. B. with NaCl in concentration isotonic to sea water. Other values are uncorrected.

with carbonated sea-water of a pH value lower than that of the medium at the stoppage of the ciliary movement. It may be concluded, therefore, that the stoppage of the ciliary movement under these experimental conditions should be ascribed to the effect of oxygen deficiency, but not to the accumulation of carbon dioxide excreted by the gill tissue. In other words, the amount of carbon dioxide produced by the gill tissue at the expense of oxygen in the normal sea-water in the vessel is far from being sufficient to affect the ciliary movement perceivably. Neither the specific action of carbon dioxide nor the increase in hydrogen ion concentration, caused by

the latter, does materially influence the ciliary movement before the lack of oxygen brings the cilia to stoppage, as the sea-water possesses a good deal of buffering action and absorbing power for carbon dioxide. The first stoppage of the cilia set in after two hours of experiment, the second took place one and a half hours after the renewal of the medium and the third cessation came in three hours after the renewal. These differences in the length of the time required for the stoppage of the ciliary movement may be ascribed to the difference of the respiratory rate of the gill tissue in different periods of experiment. It is generally known that various physiological processes are accelerated or retarded before and after the living system has undergone toxic or narcotic effects, according to their strength and period of action.

The pH values, at which the cilia stopped, also varied; the first stoppage took place below pH 8.15, the second about pH 6.8 and the third at 6.6. Such a wide range of variation in the pH value at the stoppage leads to the inference that the increase in hydrogen ion concentration in this range at most is not the dominating factor which brings the cilia to rest, under the conditions of the present experiment. This is the important point for our work on the oxidation-reduction potential above mentioned, which will be published in the near future[1].

It should also be noted that the ciliary movement could recover even after a long sojourn in the closed vessel in the experiment. The longer the sojourn in the chamber, the longer was the time required for the recovery of the ciliary movement on return to the normal sea water. This is clearly shown in Table V.

In experiment No. 3 (Table II), the medium employed was sea-water passed through with expired air, and its pH value was 7.5 at the start. At one and a half hours of experiment, the cilia were stopped and the pH value was found to be 7.25. The stoppage of the cilia is certainly due to the lack of oxygen as was evidenced by the prompt recovery of ciliary movement when the medium was replaced by sea-water with a lower pH value of 7.2. Two hours later, the pH of the medium fell to 6.95 and the cilia were brought to rest. The medium was again renewed with sea-water almost saturated with expired air and having a pH value of 6.5. Then the cilia gradually began to beat. One hour later, the movement was slow but steady, as the tension of carbon dioxide was already high. The preparation was allowed to stand until the next morning when the obser-

[1] NOMURA 1932

TABLE II.

Experiment No. 3. Temperature: 20.5–23.0°C. Sea-water passed with expired air.

Date	Time of experiment	pH of medium	Ciliary movement
Sept. 12, 1:45 p. m.	*hr. min.* 0	7.5	Active
	30	7.35	do.
	1 0	7.3	A little slackened
	1 30	7.25	Stopped
			Medium renewed, pH 7.2
	1 35	7.2	Cilia began to recover
	2 0	7.05	Active
	2 30	7.0	do.
	3 30	6.95	Stopped
			Medium renewed, pH 6.5
	3 35	6.50	Began to recover
	4 30	6.5	Slow but steady
	19 15	6.4	Stopped
			Cover glass removed
Sept. 13, 9:00 a. m.	19 45	6.8	Began to recover
	20 45	7.2	Recovered but inactive

vation was repeated and the cilia were found to have completely stopped at pH 6.4. Then the cover glass was removed, and after thirty minutes the pH of the medium gradually increased owing to the escape of carbon dioxide, and the cilia recovered slowly. After one and a half hours, the cilia showed slow but steady movement. The result of the experiment inevitably leads us to the conclusion that the cessation of the ciliary movement is due to the lack of oxygen, and not to the action of carbon dioxide. As the hydrogen ion concentration of pH 6.5 was not sufficient to arrest the ciliary movement, (Table II), an experiment with CO_2 sea water having a lower pH value, *i.e.*, 6.0, was carried out (Table III). Even this pH was not sufficient to bring the cilia to rest, provided the oxygen tension was sufficient for the cilia. The pH of the medium at the start was much lower than that in the previous experiment, and the ciliary movement was inactive from the beginning. But the movement continued for three hours in the first trial, and for four hours in the second trial after the renewal of the medium. The reason why it required a longer time to stop the cilia in the second trial than in the first trial may be

TABLE III.

Experiment No. 5, A. Effect of CO_2 sea-water. Temp. 20.0–21.3°C.

Time of exper. hr.	min.	pH of medium	Ciliary movement
	0	6.0	Inactive
	30	6.0	Much slackened
2	0	5.95	Feeble
3	0	5.9	Stopped
			Medium renewed, pH 6.0
3	5	6.0	Recovered
3	35	5.95	Inactive
4	0	5.9	do.
4	30	5.9	do.
5	0	5.9	Much slackened
5	30	5.9	Feeble
7	30	5.9	Stopped

that in the second trial the ciliary movement was decreased, the respiratory rate was also diminished, and the exhaustion of oxygen, which limits the ciliary movement, was retarded. The pH 6.0 of the carbonated sea-water at 20°C. corresponds to the CO_2 tension of 5/100 of an atmosphere[1]. This is equal to that of alveolar air. This pH value was never reached at the stoppage of the cilia, when the experiment was started with the normal sea-water. As this pH value was not sufficiently effective, another

TABLE IV.

Effect of carbonated sea-water. Temp. 21.2–21.3°C.

Time hr.	min.	pH of medium	Ciliary movement
0	0	5.0	Stopped instantly and completely
	30	5.0	Stopped
			Cover glass removed
1	30		No recovery
		—	Medium replaced with fresh sea water pH 8.2*
2	37	8.2*	Prompt recovery

*pH values corrected for salt error.

[1] McCLENDON and MEDES, 1925, p. 214.

experiment with carbonated sea-water of pH 5.0 was made (Table IV).

It should be mentioned, by the way, that the removal of the cover glass alone is not sufficient to admit the air rapidly into the medium and to remove the excessive carbon dioxide. The rate of diffusion of carbon dioxide in the medium seems to be rather slow. Renewal of the medium

TABLE V.

Time of exposure to CO_2-sea-water, pH 5.0	Time required for recovery of ciliary movement after return to normal sea water, pH 8.2*
min.	min
10	1
30	8
60	13

*value corrected for salt error

with fresh sea-water, on the contrary, permits prompt recovery. Toxic action of carbon dioxide is, however, indicated. A brief experiment, therefore, was made to see the relation between the time of exposure to carbonated sea-water and the time required for recovery of ciliary movement after the return of the gill tissue to normal sea-water (Table V).

The longer the sojourn in carbonated sea-water, the longer was the time required for recovery.

Effect of hydrochloric acid.

Bearing in mind that the specific narcotic action of carbon dioxide and the difference between the penetrative power of weak and strong acids, it seemed us interesting to compare the effect of the former with that of the latter kinds of acid. The pH value of the medium acidified to pH 5.0 with hydrochloric acid tended to rise during the experiment (Table VI). The medium had, therefore, to be renewed from time to time to keep it at a given low pH value. The pH of the medium rose considerably in a quarter or two, and pH 5.0 at the beginning of the experiment was not sufficient to bring the cilia to prompt stoppage. Here we clearly observe the difference of the action of carbonic acid and hydrochloric acid at the same pH value. This is certainly due to the greater penetrative power of the former.

As pH 5.0 was not sufficiently effective to arrest the cilia, a series of

TABLE VI.

Experiment No. 7. Effect of HCl. Temperature: 20.6–21.2°C.

Preparation A			Preparation B		
Time	pH of medium	Ciliary movement	Time	pH of medium	Ciliary movement
min.			*min*		
0	5.0	Inactive	0	5.0	Inactive
		Medium renewed			Medium renewed
10	5.0	Inactive	10	5.0	Inactive
25	5.4	do.	25	5.4	do.
		Medium renewed			Medium renewed
30	5.0	Inactive	30	5.0	Inactive
60	5.4	do.	55	5.7	do.

experiments were carried out with the lower pH values down to 3.4 (Table VII) to determine the critical point at which the difference in pH value clearly manifests itself in depressing the ciliary movement.

TABLE VII.

Experiment No 8. Effect of HCl.

pH of medium	Temperature	Ciliary movement
4.6	20.4	Somewhat slackened
4.4	,,	Inactive
4.0	,,	Inactive, but still continues for thirty minutes
3.6	20.1	Instantly stopped
3.4	,,	do.

The ciliary movement is affected by the hydrogen ion concentration of pH 4.6, but the movement continues at this pH for a long time, and in the range pH 4.0 to 4.4 the effect of hydrogen ion is more pronounced, but it affects the cilia very gradually and the movement is stopped after thirty minutes of exposure at pH 4.0. Below pH 3.6, however, the effect is quite immediate, and the cilia are arrested instantly. We may, therefore, locate the critical pH value between 3.6 and 4.0, *i. e.*, 3.8 approximately.

Effect of phosphoric acid.

The different pH values as given in Table VIII were examined with sea-water acidified with phosphoric acid. Two series of experiments were

TABLE VIII.

Experiment No. 9. Effect of phosphoric acid.

pH of medium	Temperature	Ciliary movement
	°C	
5.6	20.2	Somewhat slackened
5.3	,,	Slackened
5.0	20.1	Much slackened
4.6	20.7	Inactive. Stopped after 5–10 minutes of exposure
4.2	21.0	Almost stopped instantly. Completely stopped after five minutes of exposure
3.4	20.4	Stopped instantly

carried out and the results coincided satisfactorily with one another.

The critical pH value for instant stoppage of the ciliary movement in the phosphoric acid solution must lie between 3.4 and 4.2. The effect of phosphoric acid in the range of pH 4.2 to 4.6 is apparently greater than that of hydrochloric acid in the range of pH 4.0 to 4.4. In other words, phosphoric acid is much more effective than hydrochloric acid at the same pH value.

Effect of acetic acid.

The effect of acetic acid was more briefly surveyed, and the results are summarised in Table IX.

TABLE IX.

Experiment No. 10. Effect of acetic acid.

pH of medium	Temperature	Ciliary movement
5.6	19.4	Very inactive, but continues a long time
5.3	20.2	Instantly stopped
5.0	,,	do.

The movement of the cilia was instantly arrested at a much higher pH value with acetic acid than with hydrochloric and phosphoric acid. This value for acetic acid, pH ca. 5.45 approximates the critical pH value for carbonic acid, which arrests the cilia instantly at pH 5.5 ± 0.5. The effect of acetic acid in the range pH 5.3 to 5.6 is comparable to that of hydrochloric acid in the rage of pH 4.0 to 4.6.

The gill pieces were exposed to acetic sea-water of pH 5.0 for different periods of time, and the time required for recovery of movement varied with the length of time the pieces remained in the acidified sea-water (Table X).

Comparing these figures with those in Table V shows that the recovery is much more rapid with acetic acid than with carbonic acid at the same pH value, after the same period of exposure.

TABLE X.

Period of exposure to acetic acid-sea water, pH 5.0	Time required for recovery of ciliary movement on return to normal sea water
10 *min.*	Almost momentary
30	3 *min.* 30 *sec.*
60	8

ADDENDA.

The experiments above described were conducted during September of 1929, at the Asamushi Laboratory. The author had an opportunity to repeat part of the experiment with carbonic and hydrochloric acids in the spring of 1931 at the same laboratory. The temperature of the room in the former season was over 20°C., while that in the latter season was considerably low, *i. e.*, ca. 13°C.

In the latter season, the critical pH values were more precisely determined as given below.

Effect of CO_2. The stock solution of carbonated sea-water was made by saturating sea-water with carbon dioxide from KIPP's gas generator, washed on the way in solution of potassium permanganate and in sea-water. The pH of the sea-water saturated with carbon dioxide at 13°C. is about 4.8. This stock solution was diluted with normal sea-water to any desired pH values to be examined.

The tissue does not readily take up the indicators, brom cresol green and brom cresol purple, to show appreciable coloration in the short period experiment. Longer exposures in the dye solution give the tissue a considerable coloration, which, however, readily disappears on return of the tissue to normal sea-water owing to escape of dye into the surroundings. Brom cresol purple in the tissue assumes its extreme purple tone in the alkaline side, which indicates that the cell interior is approximately at pH

7.0. When the tissue is placed in the carbonated medium, the pH of the latter gradually increased by a considerable amount during the course of the experiment, probably due to dilution and buffering of carbon dioxide by the tissue and its adherent sea-water.

The critical pH, at which the ciliary movement is ceased promptly and completely in the carbonated sea-water, is 6.15 ±0.05. At the higher pH values, the movement is only slackened and continues for by far a longer

TABLE XI.

Effect of CO_2 — Temperature: 13°C.

Date	pH of medium	Time required for stoppage	Remarks
		sec.	
May 24	5.2	<30	
	5.6	<30	Indicator: Brom cresol green Tissue does not stain appreciably during period of experiment
	5.7	<30	
	5.8	<30	
	>5.8	<30	
May 24	5.9	<30	Indicator: Brom cresol purple
	6.0	<30	
	6.1	<30	
May 25	6.2	<30	
	6.2		1 *min.* incomplete stop
			2 ,, almost complete stop
			3 ,, complete stop
			final pH=6.3
	6.2		1 *min.* almost complete stop, except in small portions of tissue piece
			3 ,, complete stop
	>6.2		5 *min.* partial and incomplete stoppage
			10 ,, complete stoppage
	6.3	<60	
	6.3		1 *min.* incomplete stoppage
			2 ,, almost complete stop.
			3 ,, complete stoppage
	6.4		5 *min.* movement slowed or partially stopped
			15 ,, movement much slackened, tissue coloured considerably
			[illegible] *hrs.* movement much slackened, but no definite stoppage.

time. The critical pH at the low temperature of this season was higher than at the higher temperature of the former season. The difference is by far beyond the limit of probable experimental error of any kind, and must be due to the difference of temperature in both seasons.

The influence of temperature upon the physiological effect of hydrogen ion concentration has not yet been studied satisfactorily as far as I am aware[1,2].

In the present study, it has been shown that the physiological effect of hydrogen ion concentration varies with the temperature, and a lower concentration (higher pH) suffices to bring the cilia to rest at a lower temperature. The apparent temperature coefficient of the depressing effect of hydrogen ion upon the ciliary movement is, therefore, less than unity.

Effect of hydrochloric acid. Sea-water was acidified with hydrochloric acid to various normalities and pH values. The critical point at which the cilia are stopped completely in a relatively short time, say one minute, was determined. The results are summarised in Table XII. At pH 4.0 the ciliary movement is much affected and slackened, but it continues for a long time and no definite stoppage was observed. The pH of the medium rose up meanwhile to 4.6. At pH 3.8, the cilia were completely

TABLE XII.

May 25, 1931, Temperature: 13°C. Indicator: B. P. B.

HCl in sea water	pH	Time	Ciliary movement
2.8×10^{-3} N.	3.6	1 *min.*	Stopped
2.52 ,,	3.8	1	No stoppage
		5	Mostly stopped
		10	Completely stopped
2.4 ,,	3.9	5	Active
		10	Almost stopped
		15	Completely stopped
2.35 ,,	4.0	5	Slackened, but no stoppage
		10	Advanced retardation
		15	Partial and incomplete stoppage
		25	Almost complete stoppage
		45	Stoppage partial and indefinite, pH rose up to 4.6.

1) *Cf.* COMPTON, 1915.
2) *Cf.* GRAHAM, 1931.

stopped in ten minutes. At pH 3.9, it required fifteen minutes and pH 4.0 does not cause definite stoppage until forty five minutes of exposure. The critical point may be located at pH 3.7 ± 0.1.

GENERAL REMARKS AND DISCUSSION.

The main points of the foregoing experiments are summarised in Tables XIII and XIV. It will be seen that the critical pH value which arrests

TABLE XIII.*

Acids	pH	Ciliary movement at different times of exposure							
		30″	60″	3′	5′	10′	15′	30′	60′
Carbonic (ca. 20°C.)	7.5	+++	+++	+++	+++	+++	+++	+++	++
	6.8	+++	+++	+++	+++	+++	+++	+++	++
	6.5	+++	+++	+++	+++	+++	+++	+++	++
	6.0	++	++	++	++	++	++	+	+
	5.0	0	0	0	0	0	0	0	0
do. (ca. 13°C.)	6.4	+	+	+	+	+	+		
	6.3	+	±	0	0	0	0		
	6.2	+	+	0	0	0	0		
	6.1	0	0	0	0	0	0		
	5.8	0	0	0					
	5.2	0	0	0					
HCl (ca. 20°C.)	5.0	++	++	++	++	++	++	++	
	4.6	++	++	++	++	++	++	++	
	4.4	++	++	++	++	++	++	++	
	4.0	++	++	++	++	++	++	++	
	3.6	0	0	0	0	0			
	3.4	0	0	0	0	0			
do (ca. 13°C.)	4.0	+++	+++	+++	+++	++	±	±°	
	3.9	+++	+++	+++	++	±	0		
	3.8	++	++	++	±	0	0		
	3.6		0	0	0	0			
Phosphoric (ca 20°C.)	5.6	+++	+++	+++	+++	+++	+++	+++	
	5.3	+++	+++	+++	+++	++	++	++	
	5.0	++	++	++	++	++			
	4.6	++	++	++	+	0			
	4.2	+	+	+	0	0			
	3.4	0	0	0	0	0			
Acetic (ca. 20°C.)	5.6	+	+	+	+	+	+	+	
	5.3	0	0	0	0				
	5.0	0	0	0	0				

*The main purpose of the table is to show the time of complete stoppage of the cilia. The relative grades of ciliary motility can be compared only within the same series of experiments (with the same acid at the same pH). Comparison beyond this scope is not always valid.

the cilia in one minute is higher for carbonic acid at the lower temperature than at the higher temperature, and the difference is not evident for hydrochloric acid. The data in this respect are not complete, but the difference between the two acids at different exposures is recognizable. Here we have to deal with the influence of temperature on the effect of hydrogen ion concentration upon the chemical processes or colloid chemical conditions in the cell and also the influence of temperature on the permeability of the cell to the acids.

As to the changes in the critical hydrogen ion concentration of the medium as a function of temperature, there has been but little work accomplished as far as I am aware. Some authors performed experiments to determine the relation between the two factors just mentioned. They determined, however, the critical temperature of enzyme action as a function of the hydrogen ion concentration of the medium[1)2)], but not the changes in the critical hydrogen ion concentration as a function of temperature. Their data, therefore, are not complete enough to give a decisive conclusion on the latter problem, but seem to indicate that the optimum pH is decreased as the temperature is lowered,

The rise of temperature may affect both the general feature of the chemical action in the cell, supplying energy for ciliary movement, and also the particular action of hydrogen ion upon this process. If the temperature rise favours the former in a much greater extent than the latter, the result would be that higher hydrogen ion concentration, *i. e.* lower pH values, will be needed to arrest the cilia at the higher temperature. With carbon dioxide, the rate of penetration into the cell is rapid and the influence of temperature upon it may not be a preponderant factor, while with hydrochloric acid the rise of temperature may favour the permeability of the cell. The temperature coefficient of permeability is greater than unity in general, and it sometimes reaches 3 in the temperature interval from 10 to 20°C.[3)]

It is a well known fact that carbon dioxide has a specific narcotic action. The effect of temperature upon the narcotic action is different with different kinds of narcotic.

The effective concentration of narcotics is increased with temperature with ethyl alcohol, chloral hydrate and aceton, while it is decreased with

1) COMPTON, 1915.
2) GRAHAM, 1931.
3) GELLHORN, 1929.

rising temperature in the case of salicyl amid, benzamid and monacetin[1].

Adsorption, which is supposed to play a rôle in narcosis, is also affected by the changes in temperature in different ways. It is increased or decreased according to the nature of the adsorbent and adsorbend[2].

It should be such physico-chemical or colloid chemical conditions in the cell, as adsorption, surface tension, partition coefficient or other relations closely related to narcosis, that makes the critical pH value for carbon dioxide lower at the higher temperature. Experimental data are insufficient at present for further analysis of this problem.

It should also be considered, in this connection, that the solubility of carbon dioxide in sea-water is increased as the temperature is lowered, and at the same tension of CO_2 the pH of the sea water is lower at the lower temperature than at the higher temperature. For instance, at partial pressure of 0.01 atmos. of CO_2, the pH value of the sea-water is about 6.6 at 10°C. while it is about 6.8 at 20°C.[3]

In other words, the tension of carbon dioxide in sea-water at a given pH value is higer at a higher temperature than at a lower temperature.

HAYWOOD recently studied the effect of CO_2 tension upon the inhibiting ciliary movement of the gill of *Mytilus* and concluded that the length of time that cilia continue to beat in acidified sea-water depends to some extent on the pH value of the solution, but to a greater extent on its carbon dioxide tension. The results of this author show that the critical pH at which the carbonated sea-water stops the cilia at once is as follows: —

At low tension of CO_2	pH 3.7
At moderate „ „ „	4.4
At high „ „ „	5.9

The last value is well in accord with the results of our experiment, in which the pH of the medium was lowered by high tension of carbon dioxide.

On adding acids to sea-water, the carbon dioxide tension in the latter will be raised, and the excess of tension in sea-water over that in the air will be released by the escape of CO_2, when the equilibrium between the sea-water and the air is established. In our experiments with acids other than carbonic acid, the acidified sea-water was well shaked with the air and was allowed to stand for a good deal of time, and the tension of

[1] HÖBER, 1926, p. 586.

[2] FREUNDLICH, 1923, pp. 129, 154–6, 159, 302.

[3] McCLENDON and MEDES, 1925, p. 214.

CO_2 in the sea-water may safely be assumed to be in equilibrium with that of the air.

The difference between the effect of different pH values as caused by carbonic or acetic acid appears rapidly, while it appears rather slowly in the case of inorganic acids. This is in accord with the fact that organic acids in general penetrate the cell rapidly and inorganic acids penetrate slowly.

It has been supposed by some authors that in bivalve molluscs, such as *Mytilus*, the accumulation of carbon dioxide in the mantle cavity inhibits the ciliary movement and thus cuts down the oxygen consumption, when the animal is subjected to unfavorable conditions of respiration. And the inhibition of normal ciliary activity is supposed to serve for retaining the reserve of oxygen supply[1]. GRAY also writes " It is well known that many bivalve molluscs are capable of living for many hours with their valve tightly closed: if a specimen of *Mytilus* is removed from the sea-water and left for two or three hours, it can be shown that the concentration of CO_2 in the medium bathing the mantles and gills in sufficient to inhibit all ciliary movement. On washing the tissues in clean sea-water acitive movement is soon resumed. It is more than likely that the period during which *Mytilus* is normally uncovered at low tide is sufficient to produce enough CO_2 to inhibit its cilia, and thereby reduce the O_2 requirements of the animals "[2].

This supposition appears quite probable at the first glance, but it seems unjustifiable judged from our experimental results. It seems likely that it is not the accumulation of carbon dioxide, but the lack of oxygen, that brings the ciliary movement to rest. It has been shown that the last statement is true at least in our experiment on the cilia of the *Pecten* gill.

COLLIP[3] has shown that the rate of CO_2 production in *Mya arenaria* at 14°C. varied from 1.40 ccm. to 5.78 ccm. per 100 gm. drained clam tissue per hour, under the aerobic conditions, and that the animal produces CO_2 at the uniform rate of 1.24 ccm. on average under the anaerobic conditions, in oxygen-free sea-water, for over 140 hours, other conditions remaining the same as above.

Moreover, NOZAWA[4] has shown that the oyster carries on anaerobic respiration when the oxygen tension is decreased, and the respiratory

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1) GRAY, 1928, p 108.
2) GRAY, *ibid.* p. 79–81.
3) COLLIP, 1921.
4) NOZAWA, 1929.

quotient will be more and more increased. When the oxygen concentration in the medium is diminished down to 0.035 volume per cent. the respiratory quotient is over 6.0. GALLTSOFF and WHIPPLE[1] have shown quite recently that changes in pH values of the medium such as can be brought about by the oyster under the conditions of the experiments do not alter its metabolic rate, and the shell of the oyster acts as an efficient buffer, preventing the lowering of the pH below 6.0. Low oxygen tension and variation in the pH values of water from 8.2 to 6.6 had no effect on the rate of flow of water through the gills.

The sea-water contains about 6 ccm. of oxygen per liter. When this amount of oxygen is completely exhausted by respiration, about the same volume or less of carbon dioxide, if respiratory quotient is less than unity, will be evolved. This amount of CO_2 corresponds to $6/2240$ M. = ca. 0.5×10^{-3} N. Even hydrochloric acid of this normality in sea-water depresses the pH of the latter only to ca. 6.9[2,3].

As carbonic acid is much weaker than HCl, the former in the same concentration can not lower the pH of sea-water to such an extent. According to NOZAWA[4] the pH of sea-water, which was 8.2 initially, was lowered to about 7.95 by the increase of CO_2 content by 6 ccm. per liter. Such pH value is much higher than the critical pH for CO_2 obtained in our experiment. During the period of low tide, in which bivalves like *Mytilus* are exposed to the air, much CO_2 will be produced by respiration. It must not be the effect of CO_2, however, that first comes into play to arrest the cilia, but the lack of oxygen, as we have shown above. The results of our experiment on the intracellular oxidation-reduction potential will appear in another place, as mentioned above, and it may be added here in passing that the gill tissue of *Pecten* can reduce methylene blue and thionin not only in the cell interior but also in the respiratory medium. This indicates that the gill tissue can utilise oxygen of extremely low tension which can not be detected by the ordinary analytical method. The normal reduction potential of methylene blue[5] is $+0.01$ volt at pH 7.0, and -0.025 at pH 8.1, at which ciliary movement is arrested in the normal sea-water from the lack of oxygen, and this corresponds to rO 48.9 or rH 16.8 at pH 8.1. The symbol rH

[1] GALLTSOFF and WHIPPLE, 1930.
[2] KOKUBO, 1929, p. 262.
[3] GRAY, 1928, p. 80.
[4] NOZAWA, 1929.
[5] RAPKINE, Strüyk et WURMSER, 1929. *Cf.* MICHAELIS, p. 79.

refers to the negative logarithm of the partial pressure of hydrogen in atmos., which is in equilibrium with the reversible oxidation-reduction system under consideration, and the rO refers similarly to the negative logarithm of the partial pressure in atmos. of the oxygen in equilibrium with the system. The rH and rO values can be calculated from the observed data by the formula given in MICHAELIS' book (pp. 82–88), and are theoretical quantitative measures of the reductive or oxidising tendencies of the system in question. The lower the rH, the more reductive is the system, and the reverse is true for the rO[1].

As the number of AVOGADRO is 60.62×10^{22} (MILLIKAN, 1917), even a single free molecule of oxygen cannot exist at every moment in a liter of such medium as has rO 48.9 at pH 8.1. The cilia, therefore, must not reserve oxygen for the general respiration of the animal.

The respiratory quotient of *Ostrea circumpicta* PILSBRY is 0.86, when the oxygen tension in sea-water is normal, but it is increased over 6.0 when the oxygen tension in sea-water is lowered down to 0.035 volume per cent., and anaerobic respiration begins early before the amount of oxygen in the medium is exhausted.[2]

In our experiment it has been shown that the cilia are stopped when the oxygen in the closed respiratory chamber is exhausted, and the pH of the medium is then lowered by about 0.1 in pH unit. Either such slight change in pH or the increase in CO_2 tension could not depress the ciliary movement materially. Moreover, the ciliated cells survived 20 hours of anaerobic condition and resumed active ciliary movement when transferred to normal sea-water. GRAY himself also admits that the ciliary activity continues for about one hour in the absence of atmospheric oxygen.[3]

As to the anaerobic respiration, it may be added that anaerobic respiration is often met with among certain marine molluscs, and the crystalline style in the alimentary canal is supposed by some authors to be a factor in anaerobic respiration.[4]

GRAY also writes that the prolonged lack of oxygen is rapidly detrimental, whereas a prolonged accumulation of CO_2 is readily reversible. This statement is probably based upon his experiment of 1924, in which he subjected the cilia in an ENGELMANN gas chamber to an atmosphere

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1) *Cf.* NOMURA, 1931.
2) NOZAWA, *loc. cit.*
3) GRAY, 1924, p. 112.
4) ROGERS, 1927, p. 240.

of hydrogen. The detrimental effect above mentioned may be ascribed to the effect of hydrogen, and not to the lack of oxygen. Our experiment also shows that ciliated cells resume acitve movement when transferred to normal sea-water even after 20 hours of anaerobic stoppage of cilia.

The critical pH value (Table XIV), which suffices to arrest cilia within a given time, is highest for carbonic acid and the order of the efficiency of the acids is as follows: —

$$H_2CO_3 > CH_3COOH > H_3PO_4 > HCl$$

This order is just the reverse of the order of toxicity of anions given by R. S. LILLIE for the gill of *Mytilus*.[1]

TABLE XIV.

Critical pH values for stoppage of cilia.

Acids		pH for stoppage in 1 *min.*	pH for stoppage in 10 *min.*
H_2CO_3	(20°C)	5.5 ±0.5	5.5 ±0.5
,,	(13°C)	6.15±0.05	6.35±0.05
HCl	(20°C)	3.8 ±0.2	3.8 ±0.2
,,	(13°C)	3.7 ±0.1	3.85±0.05
H_3PO_4	(20°C)	3.8 ±0.4	4.8 ±0.2
CH_3COOH	(20°C)	5.45±0.15	5.45±0.15

It seems likely that a higher pH value suffices for carbon dioxide to arrest the cilia within a given time than for acetic acid, meanwhile the recovery of movement after carbonic stoppage at pH 5.0 requires a longer time than after acetic stoppage at the same pH and of the same duration of exposure (Tables V & X). GRAY remarks that, " it would seem that the length of time required for recovery in sea-water is not dependent upon the length of exposure to acid solution, and it would seem that the acid produces its maximum reversible effect almost at once, after which a certain amount of regulation takes place within the cell, so that the cell exposed to acid solution of thirty minutes recover just as quickly, if not more so, as cells exposed to the acid for only two minutes."[2]

His results seem contradictory to ours at a first glance. But it may be reconciled if we take into account the strength of acids employed. He

[1] R. S. LILLIE. *Cf.* HÖBER, 1926, p. 655.
[2] GRAY, 1920, p. 355.

used the mixture of 50 ccm. sea-water + 1.26 ccm. n/10 HCl. The pH of this mixture must lie between 3.8 and 4.2, according to the figures in his Table I. Hydrochloric acid at this pH is very strong, and the penetration may have been very rapid, and the difference of longer and shorter exposures would not appear, for the acid would have exerted its maximal action as soon as it entered the cell, as GRAY supposes. In our experiment, however, the acids used are carbonic and acetic, which are supposedly less toxic, and moreover the hydrogen ion concentrations were not high. The effect of these acids would be rather mild, and may have advanced gradually, so that the difference in time of exposure came out in the result.

Our experiments, at any rate, clearly show that the longer the exposure to acid sea-water of pH 5.0, the longer time it requires for recovery of ciliary movement on return to the normal sea-water. GRAY[1] points out the individual variations in susceptibility to acid sea-water and also in restorative power of the gill of *Mytilus*. We did not study systematically this point, but it may be said that we did not notice such variations in *Pecten* gill that might have brought about irregularities in results of experiment as far as we are concerned. *Mytilus* belongs to the littoral fauna and is uncovered normally for a long period of time at the low tide.[2]

Organisms under such circumstances are subjected to extreme changes in physico-chemical conditions of their environment,[3] and the changes must not be uniform according to the position of the individual animals in their habitat. This may result in the individual variations in their physiological state. *Pecten yessoensis* JAY, on the contrary, lives in the calm sea bottom, and is not subjected to severe changes of environment. The habitat is free from tidal disturbances. This may be the reason why the individual variations in our material is so slight as would not interfere with the result. Our material was supplied by the courtesy of the Fisheries Association of Tutiya from the culture bed of *Pecten*, belonging to the association just mentioned. The animals were of uniform size and in apparently satisfactory condition.

The critical value for HCl, which stops the cilia of *Mytilus* instantly, is about 1.12 ccm. of n/10 HCl + 50 ccm. sea-water. The pH of this

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[1] GRAY, 1920, Table IV.
[2] GRAY, 1928, p. 79.
[3] *Cf.* NOMURA, 1930.

mixture should be between 4.2 and 5.5.[1] The corresponding value in our result is a little lower than his. This may, however, be ascribed to the specific difference of the animals.

GRAY subjected the gill tissue, which had been previously stained with neutral red, to VAN'T HOFF solution acidified to pH 3.4 with hydrochloric acid. The tissue did not show colour change promptly. But in the solution acidified with organic acid it instantly changed the colour from dull brick red to one of brilliant red, indicating that the acid entered the cell immediately. We did not test the pH of the cell interior with each acid. It was necessary for our purpose, as was stated above, however, to see the hydrogen ion concentration of the cell interior just at the time the cilia are stopped by the lack of oxygen, or possibly by carbonic acid produced at the expense of oxygen in the respiratory medium.

Phenol red does not readily stain the cell intensely in a short stay in the solution, but longer exposure gives the tissue sufficient tint to judge the intracellular pH value, which was found to be pH 7.0 No sign of decrease in pH value was perceivable when the cilia stopped anaerobically in the closed respiratory chamber.

The colour change which GRAY observed in the gill of *Mytilus*, would apparently have been caused by strong acidity of the medium (HCl, pH 3.4). In our experiment, the pH of the exterior medium was lowered only by 0.1 pH unit when the ciliary movement was anaerobically stopped. As the initial pH of the normal sea-water was 8.2, the outer pH is still much higher than the intracellular pH. The constancy of the intracellular pH values under modified external conditions, if not extremely modified, has been reported by various authors.[2]

GRAY 1922 gives the following values as the critical pH for acids, which suffice to arrest cilia in one minute: —

HCl	3.4	Oxalic	3.1
H_2SO_4	3.1	Formic	4.0
HNO_3	3.1	Acetic	4.8
Citric	3.4	Butyric	5.2

These values are lower than the values in our results. If we allow the salt error of 0.2 in our determination of pH, the value for HCl is coincident, but our value for acetic acid is still much higher than the value given by him.

1) GRAY, 1920, Table I.
2) *Cf.* PFEIFFER, 1927, p 453.

As to the intracellular pH, various values have been reported. Some are higher than the neutral point, some are lower. PANTIN[1] studied recently the physiology of the marine amoeba, and found the pH of the endoplasm and ectoplasm of a progressing animal to be 7.6 and 7.2 respectively, and the difference between the ecto- and endoplasm during the resting tended to be rather less than during the progression. CHAMBERS and POLLACK[2] report that the intracellular reaction of the living sea urchin egg lies at pH 6.7, whereas VLÈS and VELLINGER[3] estimated the intracellular pH of *Arbacia* egg to be between 5.2 and 5.9. These values of the marine eggs are rather low, and it is supposed that the low pH values are due to the accumulation of carbon dioxide produced by active developmental processes.

It has been shown by FAURÉ-FREMIET[4] that the egg cell of *Sabellaria* undergoes changes in intracellular pH values during its development. When the egg is layed, its interior pH is $\leqq 7$; after 25 minutes when the germinal vesicle dissolves, pH$=10$; after 40–50 minutes when the egg cell is in metaphase of division, pH $\leqq 12.0$.

In the eggs in general, metabolism is very active and the carbon dioxide may be accumulated in the cell and also around the cell, as the rate of diffusion is relatively very slow in comparison with the rate of CO_2 production. The eggs are immotile, and CO_2 may accumulate around them to a considerable amount. In the case of ciliated cells, however, the sea-water bathing the cells is continually streaming as far as the cilia are beating, and CO_2 is carried away as soon as it is excreted. As the direct surroundings of the ciliated cells thus have relatively low tension of CO_2, the cell interior, which is in equilibrium with its surroundings, should also have a relatively low tension of CO_2 in comparison with the egg cell, although the former are doing much mechanical work and may have a high rate of CO_2 production. The rate of diffusion of CO_2 from the ciliated cells, however, may be much more rapid than from the egg cells. PANTIN's results above cited also show that the activity is not necessarily accompanied by high acidity, or low pH. It is probable, therefore, that the ciliated cells have a higher pH value than the egg cells as estimated by CHAMBERS and others.

YOUNGE reports that the hydrion concentration necessary to bring the

[1] PANTIN, 1924.
[2] CHAMBERS and POLLACK, 1927.
[3] VLÈS et VELLINGER, 1928.
[4] FAURÉ-FREMIET, 1924; 1925.

cilia of *Mya* to rest depends on the concentration of the hydrogen ion with which they are normally in equilibrium.[1]

It has been known that amoebae from cultures of different pH values show differnt critical pH values for the velocity of movement[2]. GRAY, as cited above, points out individual variations in susceptibility to pH changes in *Mytilus*.

Although it is desirable to compare more precisely and more extensively the results of pH work in this line by various authors with our data, it is difficult or almost impossible at present, as the different methods of pH determination employed by them comprise various inherent sources of error and difficulties in technique, which make direct comparison persistingly adhering to small differences in fractions of pH unit insignificant. Our determinations and comparisons described here, therefore, will suffice for the present purpose.

GRAY 1924 determined the respiratory quotient of the ciliated cells of *Mytilus* gill, and found it to be 0.83–0.85. Basing upon the experimental results he maintained that the energy source of ciliary movement is probably of protein nature. In his later writings, however, he remarks that the recent investigation of E. BOYLAND on the gill of *Pecten* suggests that the ciliary activity may involve the conversion of glycogen into lactic acid[3].

In this connection, it may be mentioned that glycolysis in blood is not proved below pH 6.3 and is much weakened even at pH 6.7–6.5[4].

The critical pH for carbonic acid, which arrests the cilia of *Pecten* gill lies in the neighbourhood of this value. For the other acids, the critical values are lower than for carbonic acid, but it should be noted that the penetration of those acids is much slower. The dissociation constants of those acids are higher than that of carbonic acid, and their concentrations must be relatively low at the critical pH. Moreover, it has been shown by OSTERHOUT that it is the undissociated molecules, but not ions, that penetrates the cell membrane[5].

It is conceivable, therefore, that stronger acids are much less efficient than weaker ones at the same pH. The mechanism by which carbonic acid affects the ciliary movement is not clear at present. It may be added at this point that *Arbacia* egg shows liquefaction at pH 5.1 and coagulation

[1] YOUNGE, 1925. Also cited by GRAY, 1928

[2] HOPKINS, 1926.

[3] GRAY, 1928, p 108.

[4] RONA und WILENKO, 1914.

[5] OSTERHOUT, 1926. Also *Cf.* OSTERHOUT, 192_.

at pH 4.9 in the sea-water acidified by passing carbon dioxide[1].

These pH values correspond to carbon dioxide tension of approximately 400 and 700 mm. Hg. respectively according to HENDERSON and COHEN[2]. At such a high tension of CO_2, not only the colloidal state of the protoplasm would be affected, but also chemical actions evolving carbon dioxide would undoubtedly be depressed or completely inhibited.

Much should be done to elucidate the chemical aspects of the physiology of the ciliary movement, and the author will pursue in this line, and other papers will follow the present one.

SUMMARY AND CONCLUSION.

1. Excised gill tissue of *Pecten yessoensis* JAY survives and the ciliary movement continues in the closed respiratory chamber with sea-water for a long period of time as far as oxygen in the medium is available.
2. The stoppage of the cilia in the closed chamber is due to the lack of oxygen and not to the effect of CO_2 excreted.
3. The gill tissue, after anaerobic stoppage of the cilia, can survive a long period of anaerobiosis over a night, and recovers motility on admitting air to the medium.
4. The amount of carbon dioxide evolved by the gill tissue at the expense of oxygen in the medium in the closed respiratory chamber is not sufficient to bring the cilia to rest.
5. The supposed regulative effect of carbon dioxide accumulated in the mantle cavity of bivalve molluscs upon the respiratory rate by depressing the ciliary activity seems to be untenable.
6. The critical pH values which bring the cilia to stoppage in given time of exposure were determined and compared for carbonic, hydrochloric, phosphoric and acetic acids (Table XIV).
7. The critical pH values are higher for the weaker acids than for the stronger ones.

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Befruchtung und Kernteilung bei *Coccophora Langsdorfii* (TURN.) GREV.[1]

VON

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(Mit Tafeln III–IV und 1 Textfigur)

(Eingegangen am 4. Dez. 1931)

Über die Oogenese und vorhergehende Entwicklung von *Coccophora Langsdorfii*, einer im Japanischen Meer einheimischen Art der Fucaceen, veröffentlichte schon Herr Prof. Dr. M. TAHARA ('28) einige Mitteilungen. Nach ihm treten in einem Oogonium dieser Pflanze drei einander folgende Kernteilungen auf und erzeugen acht Kerne, von denen einer in der Mitte des Oogoniums liegt und definitiv zum Eikern wird, während die anderen sieben beiseite geschoben werden und bald zugrunde gehen. In diesem Punkte weicht *Coccophora* von *Sargassum* (TAHARA u. SHIMOTOMAI, '25) und *Cystophyllum* (SHIMOTOMAI '28) entschieden ab und ist ein günstigeres Material zur Erforschung der Befruchtung als diese zwei.

In diesem Frühling veranlasste mich Herr Prof. Dr. TAHARA, die Befruchtungsvorgänge dieser Pflanze genauer zu untersuchen. Deshalb begab ich mich am 29. März nach der biologischen Station der hiesigen Universität in Asamushi.

Um entleerte Eier tragende Zweige zu gewinnen, besuchte ich fast jeden Tag Tutiya, wo diese Pflanzen am üppigsten in dieser Gegend wuchern. Nach mehr als zweiwöchiger erfolgloser Mühe gelang es mir erst am 16. April einige der gewünschten Pflanzen zu finden, gleichzeitig auch einige voll ausgereifte männliche Pflanzen, die ich alle ins Laboratorium mitbrachte. Aber es stellte sich bald heraus, dass zum genaueren Studium der Befruchtungsvorgänge auch bei dieser Pflanze künstliche Besamung ganz notwendig ist. So versuchte ich durch folgende Methode die beiden geschlechtlichen Zellen im Laboratorium austreten zu lassen. Zuerst tat ich einige gereifte weibliche Zweige in ein filtriertes, von Spermatozoiden freies Seewasser enthaltendes Gefäss hinein. Anderseits legte ich einige männliche Pflanzen in eine Schüssel, die mit feuchtem Filtrierpapier bedeckt wurde, um die Pflanze nicht zu trocken werden zu lassen. Wenn

[1] Contributions from the Marine Biological Station, Asamushi, Aomori-Ken. No. 78.

es glückte, treten die Eier und die Spermatozoiden am nächsten Tage aus den Konzeptakeln aus. Die Eier sitzen sich an der Oberfläche des Rezeptakels mit Schleim fest. Die ausgetretenen Spermatozoiden bilden auch ein kleines Klümpchen an der Oberfläche des Rezeptakels, aber wenn man dieses in Wasser taucht, lösen sich die Spermatozoiden sofort los. Also gleich nach dem Hineinstecken solcher männlicher Zweige in das Gefäss, das eine weibliche, viele ausgetretene Eier auf sich tragende Pflanze enthält, bemerkt man leicht unter dem Mikroskop zahlreiche, um die Eier aktiv schwimmende Spermatozoiden.

Die Fixierung solcher weiblicher Rezeptakeln erfolgte in verschiedenen Zeiträumen nach der Besamung. Als Fixierungsmittel brauchte ich die folgende Lösung.

Vorratslösung von Chromsäure (Seewasser 98 cc, gesättigte Lösung von Chromsäure 2 cc)	50 cc,
Seewasser	50 cc,
2%ige Osmiumsäure	5 cc,
Eisessigsäure	2.5 cc.

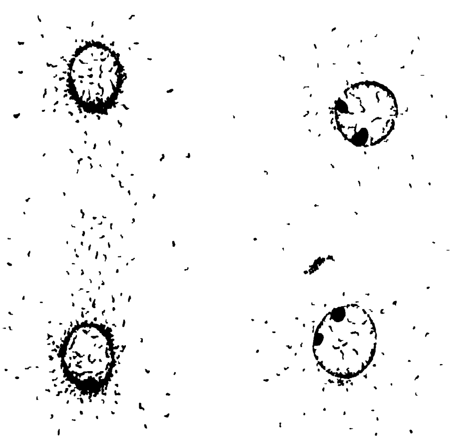
Die Fixierung dauerte meistens 3 Stunden. Die Mikrotomschnitte waren 9–10 μ dick. Zur Färbung wurde HEIDENHAINS Eisenalaun-Hämatoxylin benutzt.

Die ausgetretenen Eier sind meistens rundlich oder ellipsoidisch und enthalten zahlreiche Chromatophoren um den zentralen rundlichen Kern. Eine Stunde nach dem Hinzufügen der Spermatozoiden beginnt der Spermakern in das Ei einzutreten (Fig. 1). Dann nach einer halben bis zu einer Stunde kommt der Spermakern allmählich in die Nähe des Eikerns (Fig. 2, 3). STRASBURGER (1897) beschreibt die Befruchtungsvorgänge von *Fucus vesiculosus* etwas genauer. Nach ihm wird der Spermakern dieser Alge im Zytoplasma des Eis in den Präparaten ganz dunkel gefärbt und scheint in der Struktur fast homogen zu sein. Dies ist auch der Fall bei *Hesperophycus Harveyanus* (WALKER, '31). Bei *Coccophora* ist aber die innere Struktur des Spermakerns etwas deutlich, d. h. ein Kernkörperchen und Chromatingerüst lassen sich fast immer klar wahrnehmen. Der Spermakern ist grösser als die Chromatophoren, und sie zu unterscheiden ist gewöhnlich nicht schwierig. Der Eikern ist chromatinarm und wird in den Präparaten nur schwach gefärbt. Drei Stunden nach der Besamung kommt der Spermakern in Kontakt mit dem Eikern und verschmilzt bald mit ihm. Er flacht sich dabei zu einem linsenförmigen Körper ab (Fig. 4, 5, 6). Aber in einer halben bis einer Stunde wird er gänzlich desorganisiert (Fig. 7, 8, 9, 10, 11). In diesem Zustand bemerkt man meistens

zwei Kernkörperchen, von denen wahrscheinlich das eine vom Spermakern und das andere vom Eikern herstammt. STRASBURGER (1897) bemerkt beim ähnlichen Fall von *Fucus*, dass das Kernkörperchen des Spermakerns an Grösse, Schärfe des Umrisses und Intensität der Färbung dem Kernkörperchen des Eikerns nachzustehen pflegt. Solche Unterschiede konnte ich aber bei *Coccophora* nicht erkennen. Diese zwei Kernkörperchen nehmen danach an Grösse ab, der Eikern aber zu (Fig. 12, 13, 14).

Mit dem Fortschreiten der Stadien gestalten sich die Chromosomen allmählich aus dem Kerngerüst. Sie sind zuerst im Kernraum unregelmässig zerstreut (Fig. 13, 14). Leider konnte ich die Spindelbildung nicht verfolgen. Zentrosomen, die bei anderen Gattungen der Fucaceen, d. h. *Fucus* (YAMANOUCHI, '09), *Sargassum* (TAHARA u. SHIMOTOMAI, '25, OKABE, '29, '30) und *Cystophyllum* (SHIMOTOMAI, '28) entdeckt wurden, konnten bei *Coccophora* nicht beobachtet werden, worauf schon TAHARA bei der Untersuchung der Reduktionsteilung dieser Pflanze aufmerksam gemacht hat.

Das 18 bis 22 Stunden nach der Vermischung der beiden Geschlechtsprodukte fixierte Material zeigte verschiedene Figuren der Metaphase (Fig. 15, 16, 17). In Fig. 15 ist die Spindel eines Keimkerns in Seitenansicht gezeichnet. Bei vollständiger Metaphase löst sich die Kernmembran gänzlich auf, und die kleinen, stäbchenförmigen Chromosomen verteilen sich regelmässig auf einer Äquatorialplatte. Es gelang mir manchmal, die Polansicht solcher Kernplatte zu sehen, wo ich mit Sicherheit 64 Chromosomen zählte (Fig. 17). Dies ist die diploide Chromosomenzahl dieser Pflanze, da bei der Reduktionsteilung dieser Pflanze TAHARA ('28) 32 gezählt hat. Bei der Anaphase rücken die beiden Längshälften der Chromosomen regelmässig in die entgegengesetzte Richtung auseinander (Fig. 18). Und am Ende der Telophase kommen gewöhnlich zwei Kern-



a b

Textfig. 1. a. Frühere Telophase.
b. Spätere Telophase. Je zwei Nukleolen in jedem Kern.
Vergrösserung. 1100.

körperchen zum Vorschein (Textfig. 1, a, b), wie beim Fall von *Sargassum Horneri* (OKABE, '30).

Die vorliegende Arbeit wurde auf Anregung von Herrn Prof. Dr. TAHARA unternommen, dem Verfasser für seine vielseitigen Ratschläge zu grossem Dank verpflichtet ist.

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TAFELERKLÄRUNG

Alle Figuren wurden mit dem LEITZschen Objektiv, Ölimmersion 1/12, ZEISSchen Okular ×17, mit Hilfe eines ABBÉschen Zeichenapparats gezeichnet. Vergrösserung 1900.

TAFEL III.

Fig. 1. Der Spermakern in Kontakt mit dem Ei. 1 Stunde nach der Besamung.

Fig. 2, 3. Der Spermakern auf halbem Weg zum Eikern 1 bis 2 Stunden nach der Besamung.

Fig. 4. Die Verschmelzung des Spermakerns mit dem Eikern 3 Stunden nach der Besamung.

Fig. 5, 6. Der in den Eikern eindringende Spermakern.

Fig. 7, 8, 9. Die Desorganisation des Spermakerns im Eikern

Fig. 5–9. 3 bis 4 Stunden nach der Besamung

TAFEL VI.

Fig. 10, 11. Der Ruhekern nach der Befruchtung.

Fig. 12. Die frühere Prophase der ersten Teilung des Keimkerns.

Fig. 13, 14. Die spätere Prophase.

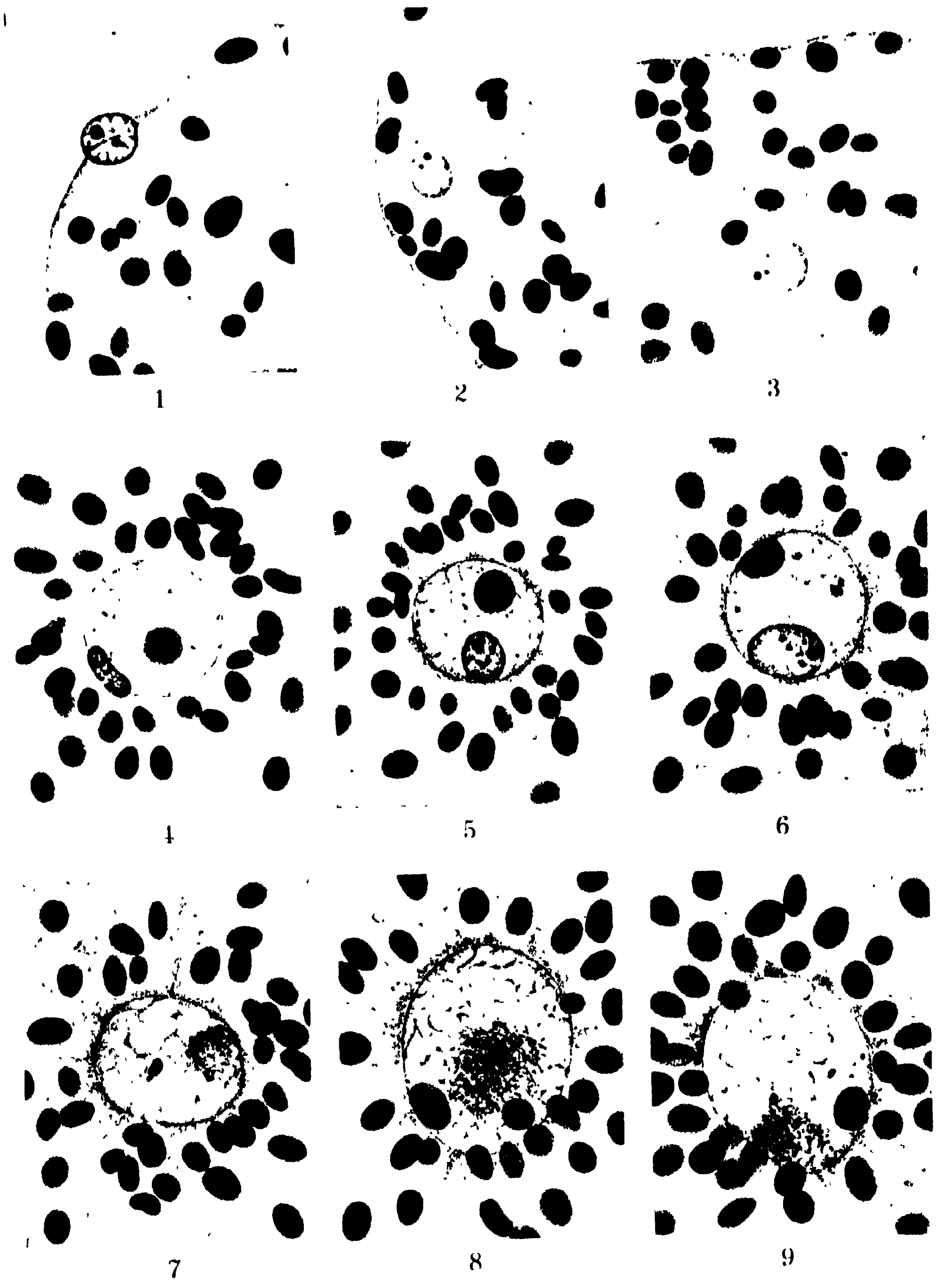
Fig. 15. Die frühere Metaphase.

Fig. 16 Die Seitenansicht der Spindel.

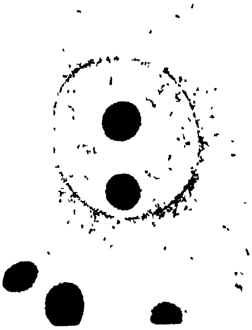
Fig. 17. Dieselbe in Polansicht, wobei 64 Chromosomen deutlich zu sehen sind.

Fig. 15–17. 18 bis 22 Stunden nach der Besamung.

Fig. 18. Anaphase.



K. Tomita: Befruchtung und Kernteilung bei *Coccophora*.



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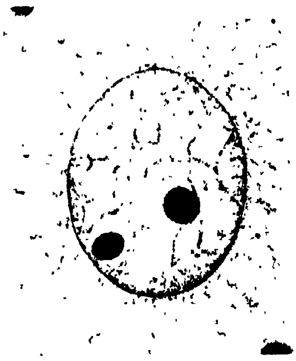

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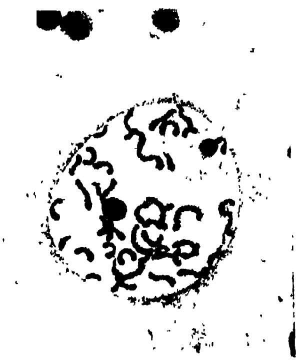

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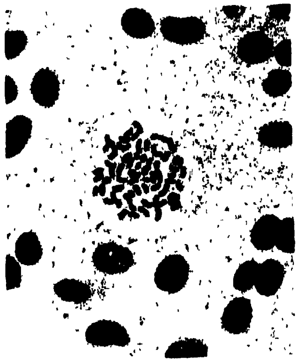

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18

K. Tomita: Befruchtung und Kernteilung bei *Coccophora*.

Hemocytopoietic Effect of Splenectomy in the Newt, *Diemyctylus pyrrhogaster.*

By

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(With Pls. V-VI, and 4 text-figs.).

(Received Dec. 9th, 1931).

In the present paper the hemocytopoietic effect of the splenectomy upon the Japanese newt, *Diemyctylus pyrrhogaster*, is reported. As is seen in my foregoing paper, the extirpation of spleen caused severe anemia; then recovery took place and the anemia completely returned to normal within 14 to 16 days. There must have occurred, therefore, the compensatory hemopoiesis in some part of the body. Where would this have occurred? On this interesting problem, the contributions working with the mammals are found very abundant. Of the observation made on the lower vertebrate, however, so far as I am aware, I can refer to a few reports only, as those of DAIBER, JORDAN and SPEIDEL, and WITUSCHINSKI. From the standpoint of blood formation, the splenectomy on the amphibia is especially interesting. For the spleen is the sole erythrocytopoietic centre in the mature animals of this group, which have no red bone marrow. I took note on the changes which occurred in several organs and general circulation, following the extirpation of spleen. I have set out below the observation.

EXPERIMENTAL METHODS.

The experiments were performed upon full grown animals collected chiefly during the early summer months at Matuyama. About 160 animals were splenectomized after about twenty days of collecting, and each of the 20 animals was kept in an aquarium. Small earthworms and boiled flesh of fishes were given to the newts every two or three days throughout the experiments. The 80 control animals were fed and kept in the same condition as that of the experimental animals, so far as it was possible.

The splenectomized newts were killed with the controls at an interval of about one week, extending from one to forty weeks.

Eosin-haematoxylin or eosin-azure stain was used for tissues after fixation

in HELLY's fluid. For blood smears and organ smears, MAY-GIEMSA's stain was exclusively applied.

OBSERVATIONS.

1. Spleen.

Recently NAKAJIMA ('28) published a paper treating the comparative histology of the amphibian spleens with special reference to their seasonal variation. According to his description, the spleens of Urodels and Anuras show remarkable seasonal changes of their contents. My observation was made throughly on the specimens killed in early summer.

The normal spleen was red and vascular with great variation in size. The following facts were found by the result of the microscopic examinations of 20 normal spleens.

The capsule consists of rough and thick fibres of connective tissues. The fine and waved elastic fibres are also found in this portion. Trabecula is not remarkable. The reticular cell is of a polygonal shape and has an eccentric nucleus.

The percentage of the free cell content of 20 specimens is seen in Table I.

TABLE I.

Free cells in the spleen.

Free cells	Erythrocytes	Lymphoid cells	Eosinophils	Basophils	Neutrophils	Pigment cells
Percentage	20.0%	73.0%	1.0%	0.5%	0.6%	5.9%

2. Spleen regeneration.

In more than 150 splenectomized animals killed within 40 weeks, there were only two cases of spleen regeneration. The regenerated spleens were situated under the serosa of the stomach walls in two or three small masses. Histologically, these masses were composed chiefly of the red blood cells, lymphocytes and mesenchymal elements. The removal of the spleen was made so carefully that macroscopically there was not discernible, on the cut ends of the splenic vessels, any the least remaining that might grow up into a new spleen. I suppose rather, therefore, the element

capable to reproduce spleens must, at least in some cases, be present after the splenectomy.

In literature, JORDAN and his coworker ('25) reported three in total and two doubtful new spleen formations in more than 90 splenectomized frogs. The experiment of the same authors on the American salamander, *Triturus viridescens*, was negative in all specimens. DAIBER ('07) and WITUSCHINSKI ('28) showed that the Axolotl (the larval form of *Amblystoma tigrinum*) deprived of the spleen by operation readily regenerated a new one. The difference of spleen regeneration in the Axolotl and the American salamander, according to the explanation of JORDAN, may be explained from the ground of relative age. In the former the mesenteric components still possessed the capacity for spleen formation, while in the latter the developmental period was too far advanced. In my experiment, the spleen regeneration took place in full grown animals. This fact seems to make it probable that some animals of present investigation may have been at the primitive stage of the blood formation as compared with the American salamander.

That the accessory spleen may be found upon rare occasions in the amphibia is generally accepted. I examined 80 animals especially for this purpose. There was, however, no trace of the accessory spleen.

3. Liver, granulocytopoietic shell.

As is seen in the report of JORDAN, granulocytopoiesis and lymphocytopoiesis in the salamander are normally restricted almost completely to the subcapsular region of the liver, erythrocytopoiesis and thrombocytopoiesis to the spleen. Thus the hemopoietic functions are here distinctly divided between two main loci

Beneath the capsule of the liver, there occurs a granulocytopoietic shell varying in thickness from one to eight layers of cells. Here, the reticular cells and hemoblasts are progressively transformed into various granulocytes. In the present experiment, changes in this portion following the splenectomy are coincident with those of JORDAN's experiment.

It is noticeable that the pigment-leucocytes formation in the liver capsule was observed in some operated animals of the present investigation. In some extreme cases, the liver capsule was filled almost with pigment-leucocytes. As later seen, the splenectomy frequently causes the increase of pigment-leucocytes in the general circulation. The liver capsule, therefore, may be an essential locus of the pigment cell formation. The precursor of the pigment leucocyte seems to be the reticular cells, but the

pigment accumulation in the usual granulocyte mother-cells was seen in some cases.

4. Liver, sinusoid regions.

In some cases, there appeared increased stellate cells which were much enlarged and proliferated from the wall of blood vessels. These proliferated stellate cells (histiocytes) flock together into a cell mass, making islands in the sinusoid cavity. These changes were observed within 2 weeks after the operation (Pl. V, Fig. 2). While the stellate cells are normally negative or very slight in the iron reaction with the Berlin blue, those of operated animals were almost always positive in the reaction. This reaction may be due to the ingestion of the blood pigment, chiefly the débris of senile erythrocytes. The stellate cells, therefore, are also a source of brown pigment cells of liver and general circulation.

The number of liver pigment cells is much variable with the seasonal and nutritive condition. But generally speaking, there seems to be evident, more pigment cells in the operated animals than in the controls, while both were kept under the same condition and feeding. The number of pigment cells in an unit area (ca. 180 mm^2) of the livers of ten operated and ten control animals are as follows.

TABLE II.

Nos. of pigment cells in about 180 mm^2 of liver. Every figure is an average of 10 counts.

											Average
Normal	42	36	31	26	21	20	18	18	14	13	23.9
Splenectomized	72	56	53	46	42	32	31	26	19	18	39.5

In the case of severe anemia, the dilatation of liver capillaries was usually observed and intravascular differentiation of hemoblasts was distinctly seen in these portions (Pl. V, Fig. 3).

5. Hepatectomy with the coincidental splenectomy.

As is mentioned above, the spleen and liver are the chief loci of the hemopoiesis in the salamander and frog. The total splenectomy with coincidental total hepatectomy, therefore, means practically the loss of all hemopoietic organs. These animals died within about five days. The

partial hepatectomy with the coincidental total splenectomy produces no noticeable changes in the appearance of animals. These operated animals lived until they were killed during 1–8 weeks for the study of the remaining liver tissues.

Recently JORDAN and BEAMS made the simple hepatectomy in the salamander. According to their description, the partial hepatectomy showed no microscopic evidence of regeneration. The remaining lobe, however, was somewhat enlarged by the 27th day, and had apparently satisfactorily effected the histologic and physiologic regeneration. The granulocytopoietic capsule was thickened and very active mitosis occurred in this portion. Since the hepatectomy was made in parallel with splenectomy, some differences must be seen in the present case as compared with the results of JORDAN's investigation. This expected difference was the stimulation of stellate cells in the remaining lobes. As I said already, the stellate cells of the liver show the active proliferation and pigment ingestion in the splenectomized animals. In the present case, the stellate cells react also in the same manner, but more intensively than in the case of simple splenectomy.

The partial hepatectomy causes, of course, the high hyperaemia of the remaining lobe. In such a liver, the parenchyma usually becomes loosened into irregular cords of cells separated by wide sinusoids. In the severest cases, the parenchymal cells are so completely separated and disorderly scattered that the evidence of the cell cords can not be discerned. The liver cells, however, still appear healthy, though stained less deeply with eosin than the normal. The cells of granulocytopoietic capsule also dissociate irregularly, and occasionally completely disappear.

6. Heart.

WITUSCHINSKI, working with Axolotl, found that erythrocytopoiesis in splenectomized animals is taken over by the cells of the reticulo-endothelial system of the liver and heart; in the heart the reticulo-endothelia associated with the sponge-like structure of the muscular pillars were especially stimulated. JORDAN and SPEIDEL also reported the affirmative result on this interesting problem, working with adult salamander. The results of present investigation were also corroborative to the foregoing findings.

A reticulo-endothelial cell first hypertrophied, and rounded; then proliferated and migrated into the lumen of the heart (Fig. 1 & 2). The intensive mitosis was usually seen everywhere on the endocardinal wall. Separation from the cardinal wall followed with the formation of typical

hemoblast. The hemoblasts in the various stages of the differention were seen plentiful in the lumen of the heart (Pl. V, Fig. 4). The prolifreation

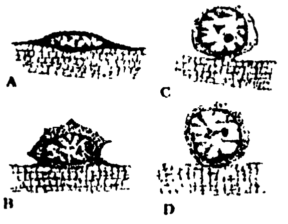

Fig. 1. Hypertrophy and separation of a cardiac endothelium.

Fig. 2. Hemopoiesis of cardiac endothelium.

of hypertrophied reticulo-endothelium began within two weeks after the operation.

7. Kidney.

According to JORDAN and SPEIDEL, the erythrocytopoiesis and granulocytopoiesis took place in the kidney of splenectomized frog, and it occurred apparently extravascularly. In the studies on the salamander by the same authors, the kidney showed no conspicuous deviation from the normal. They found, however, the evidence of marked proliferation of hemoblast in a few cases and erythrocytopoiesis in one case.

Fig. 3. Intertubular hemopoietic field of kidney. h, hemablast; m, mitosis.

In the present investigation, there was no evidence of erythrocytopoiesis in the kidney of any specimens. The marked proliferation of hemoblast, however, was seen in many cases. It set out from about 28 days after splenectomy, and continued thenceforth continuously.

Of special interest is the positive iron reaction of tubuli and intratubular contents. NISHIKAWA and TAKAGI ('19) found this reaction in the splenectomized rabbit. In the case of pernicious anemia, this reaction is also positive. The tubuli and their contents are stained in blue here and there. This reaction, having

appeared within one week after the operation and continued for about another 60 days. Such abnormal excretion of iron must be due to the absence of the spleen. As BOTAZZI pointed out, the spleen serves, on the one hand, as an essential hemopoietic organ, and as hemocatatonistic organ on the other. This function, after the splenectomy, is taken over by the liver so dissatisfactorily that a certain part of iron due to the disintegration of blood pigment may be excreted through the kidney. The liver, however, adapts itself to reserve the iron in the course of time, by means of the increase of iron ingesting capacity of stellated cells. The iron reaction of kidney, therefore, disappears or becomes less intensive in about 10 weeks after operation, contrasting with the increased reaction of liver.

8. Bone marrow.

According to the papers of JORDAN and his coworker, the extirpation of spleen caused erythro- and lymphocytopoiesis in the long bone of frog. The result on the salamander by the same authors was rather negligible from the standpoint of the blood formation. The findings in the present investigation on this point were also insignificant.

The observation was made exclusively upon the marrow of humerus and femur. The bone marrow of the normal newt is usually yellow and crowded with adipose cells, and is deficient in the vascularization. The long bones of the operated animal show a more reddish appearance in color than the normal. Microscopically the intensive vascularization is seen in the first place. These capillaries are filled with the erythrocytes. A few hemoblasts are also seen extravascularly (Pl. VI, Fig. 6). There is, however, no indication of erythrocytopoiesis in any specimen.

9. Fat bodies.

In general, the changes in the fat bodies were very similar to those of the bone marrow. The increase of lymphocytopoiesis were seen in this tissue of the operated animals.

10. Gonads.

From the standpoint of the blood formation the testes are negligible in Urodels. No difference in these regions was noted between the operated animals and the controls.

In the epithelia of ovaries, small masses of pigment cells are normally seen. In some of the operated animals there appeared to be increased deposition of these cells in the epithelia. The plasma cells were also found

in this portion mixed with pigment cells. The plasma cell will be later described in detail.

11. General circulation, blood smears.

Blood smears were prepared from every animal used for the observation above mentioned.

As to the morphology of the normal blood corpuscles of the Japanese newt, USUI ('22) has reported in detail.

In the present investigation, the differential count of leucocytes from the 40 normal animals was as follows;

TABLE III.

Differential leucocytes-count of normal newt.

Large lymphocyte	Small lymphocyte	Mono & transitional lymphocyte	Eosinophil	Neutrophil	Mast cell	Pigment leucocyte	Plasma cell	Thrombocyte
7.8%	11.7	6.3	2.4	21.7	15.8	0.9	0.5	33.9

The differential counts of leucocytes show great variation after splenectomy. I pick up here a few cases of significant variations.

TABLE IV.

Differential count of leucocyte after splenectomy.

Leucocytes (%) / Animals	Large lymphocyte	Small lymphocyte	Mononuclear & transitional lymphocyte	Eosinophil	Neutrophil	Mast cell	Pigment leucocyte	Plasma cell	Thrombocyte
90 days after splenectomy,	5.0	8.8	4.0	1 0	15.0	9.0	**30 5**	0	26.7
190 days,	5.6	9.1	3.5	1.0	**51.1**	9.1	0	0	20 7
210 days,	6.2	8.2	6.3	2.3	17.1	15.0	2.0	**15.6**	27.4
230 days,	7.7	11.4	4.8	1.4	16.6	**20.6**	4.5	3.2	29.8

a) Erythrocytes.

It is reported by many workers that hemoblasts, proerythroblasts, erythroblasts, megaloblasts, and polychromatophiles appeared in the normally

circulating blood of amphibia. These cells, however, are very small in number and their mitosis is found on very rare occasions.

In the splenectomized animals, the mitosis of erythroblasts took place extensively and all series of young erythrocytes could be found easily in the circulating blood. Thus the general circulation becomes an important locus for the multiplication and differentiation of young erythrocytes and hemoblasts after splenectomy.

According to the recent contribution of TORYU, the splenectomy upon the carrier-pigeon causes an appearance of the polychromatophiles about 0.5 to 10.0 per cent. of the total red blood cells. He states that the number of these cells shows a gradual increase with the progress of anemia up to the 13th day after splenectomy; in about two weeks, when the erythrocytes begin to increase, the polychromatophiles suddenly diminish, but never disappear throughout the entire period of anemia of the animal, following splenectomy. In my experiment, the polychromatophiles behaved also in a similar manner, but the disappearance of those cells has not occurred even after a complete recovery of anemia.

The total number of young red blood cells reached in maximum about 22% of mature ones. As is seen in my foregoing papers ('27, '30), the splenectomy causes an increased resistance of red blood cells to hypotonic salt solution and saponin. This increase of resistance seems to be due to the appearance of young forms of erythrocytes which are, of course, much more resistant than the senile on the verge of the disintegration.

The small erythrocytes, from 1/2 to 1/3 in the longer diameter compared to that of the common red blood cells, appeared as a normal element of amphibian blood. Often these cells were seen in mitosis after the splenectomy.

Anucleate erythrocytes were seen also in all specimens, though usually in small numbers. There appeared to be no significant difference in those between the operated and the control animals.

b) Lymphoid cells.

The mitosis of lymphoid hemoblast was seen in many specimens kept for more than 90 days after the operation.

c) Basophilic leucocytes (Mast cells).

As USUI has indicated, young and adult forms of mast cells are distinguished.

The evidence of the increase in number of mast cells after the

operation is rather rare, though in a few cases they exceeded 20% of the total leucocytes. The young and adult basophils exist almost equally in the splenectomized animals. Later on the degenerative processes set in the adult basophils; that is, the granules are disintegrated, the cytoplasm vacuolized, and the nucleus loses its definite structures.

FRIEDSOHN ('10) noted the presence of polynuclear basophils in the amphibian blood. JORDAN also stated the lobulated nuclear basophils as common elements of the salamander blood. I failed, however, to find such polynucleate, or lobulated nuclear basophils through all the operated and normal newts.

d) Eosinophilic leucocytes.

Any remarkable changes of the eosinophilic leucocytes were not observed. USUI reported the existence of mononuclear eosinophils in the blood of normal Japanese newt on rare occasions. These mononuclear ones were rather common in the splenectomized.

e) Neutrophilic polynuclear leucocytes.

Enormous increases of the neutrophilic leucocytes frequently occurred. The neutrophils exceeded about 50% of the total leucocytes after the removal of spleen. In the cases of such enormous increase, the anemia following the splenectomy usually did not recover; that is, the number of erythrocytes remained under about 170,000 and the erythrocytopoiesis is not found in any places. It seems to be that the newly increased neutrophils possessed many lobulated nuclei. The frequency of nuclear lobulation of 300 cells is seen in Table V.

TABLE V.

Nos. of nuclei of neutrophilic polynuclear leucocytes.

Nos. of nuclei / Frequency (%)	2	3	4	5	6	7	8	9	10	11	12	13	14
Normal	3.7	11.0	20 0	25.0	20.0	14.0	4.0	1.4	0.5	0.4	—	—	—
Splenectomized	1.7	6.0	10.0	13.3	15.0	18.6	14.0	9.3	5.0	2.7	3.0	0.7	0.7

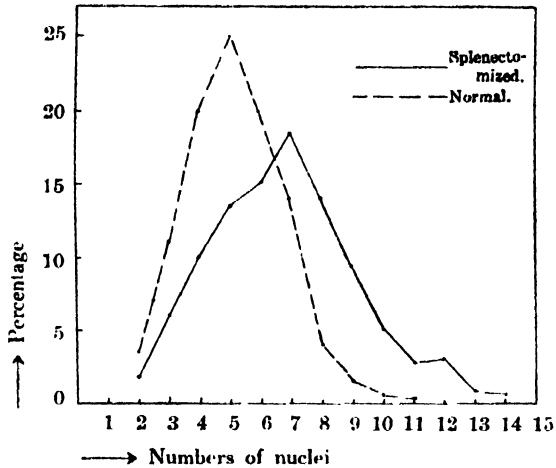

Fig. 4. Frequency of nuclear lobulation of neutrophilic polynuclear leucocytes. This figure was plotted from Table IV.

f) Thrombocytopoiesis.

The splenectomy causes the decrease of thrombocytes count in the circulating blood. Most of the splenectomized animals causes the numbers of thrombocytes to become less than 30% of the total leucocytes, while the normal was about 34% in an average of 40 animals.

As indicated already by JORDAN and his coworker, the thrombocytopoiesis after the extirpation of spleen is carried on in the general circulation. In my experiment, various stages of thromboblasts in the differentiation were also observed. The thromboblast is spherical or oval in form, and has fine azurophilic granules in cytoplasm.

g) Pigment-leucocytes.

Of the presence of special pigment leucocytes which contain brown pigments in their cytoplasms, was noticed by many workers, as GRÜNBERG ('01), FRIEDSOHN ('10), WERZBERG ('11), KIYONO ('21), USUI ('22) and so forth.

The pigment-leucocyte of the newt is oval or round in form and 14–35 μ in diameter. The nucleus is also oval or round in form and usually 1–3, but rarely 8 nuclei are seen in one cell. The chromatin network of nucleus is rather indistinct. The splenectomy causes frequently an extensive increase of pigment leucocytes in the general circulation. In

an extreme case their number exceeded 30% of the total leucocytes, while they usually remain under 1% in the normal animals.

OKAMOTO ('25), working with the toads, made an observation upon the pigment cell of the spleen and the liver. According to his description, there are two kinds of pigments in the pigment cell; one is sharp-edged and dark brown colored "lipofuscin", and the other is bluntish-edged and yellow brown colored "haemosiderin". He stated that haemosiderin was seen in all pigment cells in winter, but very scanty in summer. In the present observation the pigments of both kinds were found intermixing in the cytoplasm even in summer.

h) Plasma cells and cells with RUSSELL's bodies.

Plasma cells are very common in inflammatory areas in mammals. They are produced as histologic reaction to an inflammatory stimulus in the animal body. In the cold blooded vertebrates, the cells of this group are normally present in the circulating blood. DOWNY ('11) found plasma cells in the ganoid fish *Polyodon*, in certain frogs, and in the garter snake. JORDAN and his coworker ('24) also reported the existence of secretory cells which seem to be a developmental stage of plasma cells, in certain teleosts. They also described plasma cells of the horned toad in detail ('29). It is generally accepted that the cell with RUSSEL's bodies is also a developmental stage of plasma cells. The function of RUSSEL's bodies still remains unknown.

In a few cases plasma cells and cells with RUSSELL's bodies were seen in the normal newts, though usually small in number. They were, however, very common in the splenectomized and exceeded about 15% of the total leucocytes in a few cases. And sometimes they appeared in the ovary intermixing with pigment cells as is already mentioned.

Plasma cells are oval or round in form. Their sizes vary 14–27 μ in the longer diameter. The nucleus always became pushed to one pole and compressed into oval form. The nucleus stained deeply with the basophilic dyes but the chromatin networks are rather indistinct. The cytoplasm stained somewhat basophilic, and contained from one to four pinkish staining globules. These globules apparently represent the vacuole of the plasma cells. RUSSELL's bodies stained basophilic and took either globule, granule, or crystalloid form. The granules were occasionally agglomerated in a string.

SUMMARY.

As is seen in the preceding description, the results of the present investigation accord in general to those of WITUSCHINSKI, JORDAN and his co-workers. The summary of observations is as follows:

1. Compensatory hemopoiesis following the splenectomy took place in the general circulation, heart, liver, kidney, bone marrow, and fat body, and the general circulation was the most important locus of all.
2. New spleen formation after the splenectomy was found in two cases. These new spleens were situated under the stomach serosa.
3. Partial hepatectomy with coincidental splenectomy caused no significant changes from the standpoint of blood formation, except the thickening of granulocytopoietic capsule.
4. The proliferation of stellate cells of liver sinuses was found in many specimens. These cells ingested the senile red blood cells in their cytoplasm. The iron reaction of stellate cells was usually positive and intensive after splenectomy.
5. Iron reaction of tubule of kidney was also positive during about 10 weeks after splenectomy.
6. Various stages of differentiation of thromboblasts were seen in the general circulation of experimental animals.
7. The pigment leucocytes were found in the blood of the splenectomized in abundance (30% of the total leucocytes count), while they did not exceed 1% in the normal animals.
8. Plasma cells and cells with RUSSEL's bodies were also seen abundantly in the blood and ovary of the splenectomized.

I should like to express my thanks to Prof. Dr. S. HATAI for his kind advice and help throughout the course of this investigation.

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EXPLANATION OF PLATES

PLATE V

Fig. 1. Regenerated spleens. ×50. (2 small masses on the stomach wall.) Figure in the left hand-upper corner is the macroscopic view of the same. 3.

Fig. 2. Hemopoiesis in the hepatic sinusoid region. ×80.

Fig. 3. Intravascular formation of hemoblasts in the liver capillaires. ×80.

Fig. 4. Hemopoietic field of heart. ×60.

PLATE VI

Fig. 5. Intertubular formation of hemoblasts in a kidney. ×80.
Black portion on the right side is a mass of proliferating hemoblasts.

Fig. 6. Hemoblasts in a bone marrow. ×80.

Fig. 7. Pigment leucocytes and cells with RUSSELL's bodies.
MAY-GIEMSA stain. The drawings were made with a 90 Zeiss oil-immersion lens, and no 3 compensating ocular. ×1600.
1–6 Pigment leucocytes.
7 and 8 Cells with crystall-shaped RUSSELL's bodies.
9–13 RUSSELL-body cells with coarse and fine granules.
14 and 15 Successively older RUSSELL-body cells.
16 RUSSELL-body cell with very coarse granules.

Fig. 1.

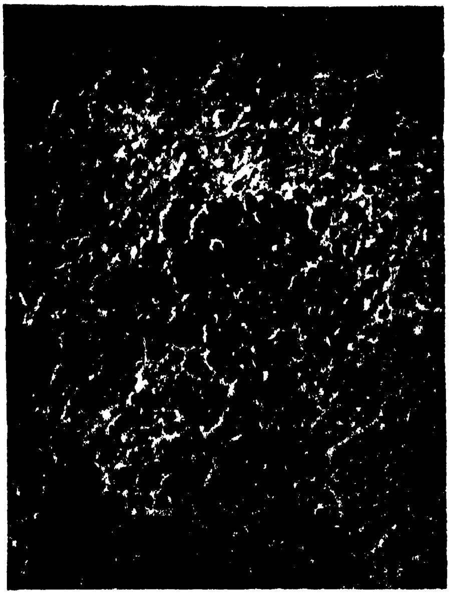

Fig. 2.

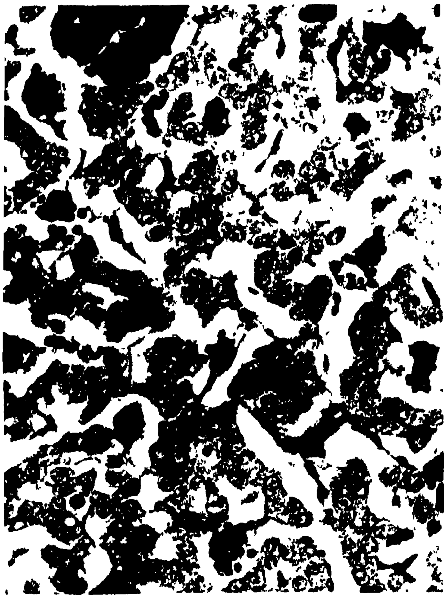

Fig. 3.

Fig. 4.

Author photo.

T. OHUYE: Hemocytopoietic Effect of Splenetomy in the newt.

Fig. 7.

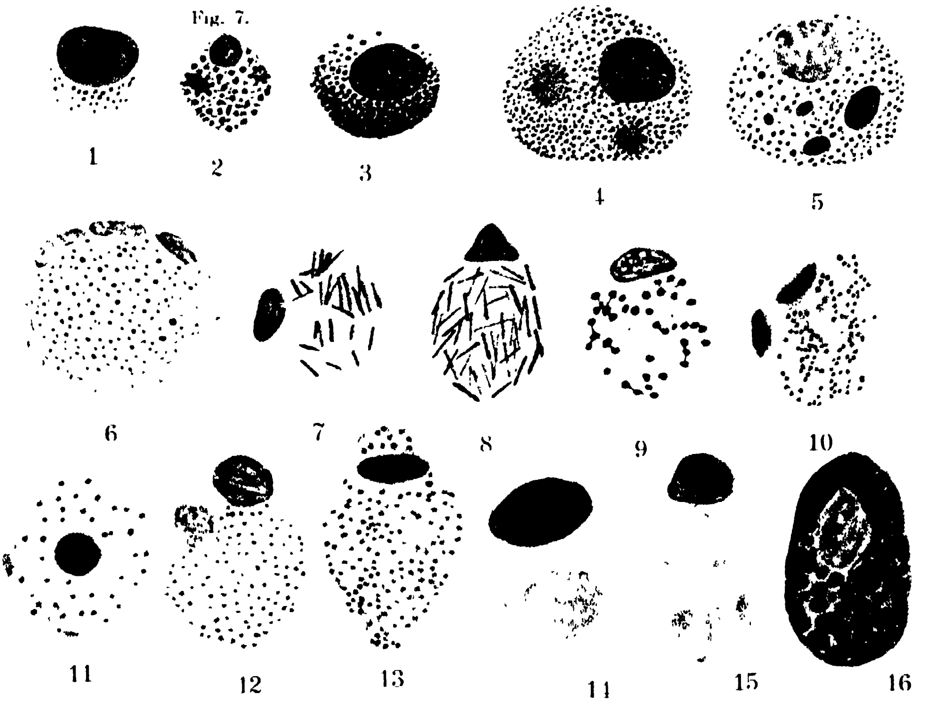

Fig. 5.

Fig. 6.

Author del. & photo.

T. Ohuye: Hemocytopoietic Effect of Splenectomy in the newt.

Mikrochemischer Nachweis von Aluminium und sein Vorkommen im Pflanzenreiche.[1]

VON

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(Eingegangen am 15. Dez. 1931)

I. Ein phytomikrochemischer Nachweis von Aluminium.

Zum mikrochemischen Nachweis einer kleinen Menge von Aluminium in Pflanzenzellen hat KRATZMANN[2] die Cäsiumprobe empfohlen, die seitdem als einzig empfindliche und brauchbare Methode gilt. Wir haben festgestellt, dass das bei der makrochemischen Analyse von Aluminium brauchbare Alizarin-S auch im phytomikrochemischen Verfahren mit gutem Erfolg anwendbar ist. Aluminiumsalze geben mit Alizarin-S ein in essigsaurer Lösung beständiger, roter Farblack, den man unter dem Mikroskop leicht in geringen Mengen entdecken kann. Das Reagens hat wenigstens den Vorteil, dass dieser Farbstoff leicht zur Verfügung steht und seine Farbreaktion leicht erkennbar ist.

Im Jahre 1915 hat ATACK[3] zuerst auf die Verwendbarkeit von Alizarinsulfosaurem Natrium zur quantitativen Bestimmung wie auch zum qualitativen Nachweis des Aluminiums hingewiesen. Später haben STOCK und seine Mitarbeiter[4] nach kleiner Abänderung der Methode gezeigt, dass dieser Farbstoff für die kolorimetrische Bestimmung kleiner Mengen von Aluminium besonders geeignet ist. Die Übertragung dieser Reaktion auf die Phytomikrochemie ist aber noch nicht erprobt worden[5], obwohl einige Forscher sie in der Tüpfelreaktion angewandt haben[6].

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1) Contributions from the Mt. Hakkoda Botanical Laboratory. No. 10.

2) KRATZMANN, E., Der mikrochemische Nachweis und die Verbreitung des Aluminiums im Pflanzenreich. Sitzungsber. d. Kais. Akad. d. Wiss. in Wien. Bd. 122, Abt. I, S. 311. 1913.

3) ATACK, E. W., A new reagent for the detection and colorimetric estimation of aluminium. Jour. Soc. Chem. Ind. Vol. 34, p. 936. 1915.

4) STOCK, A., PRAETORIUS, P. und PREISS, O., Die Darstellung des Berylliums. Ber. d. deutsch. chem. Gesell. Jahrg. 58, S. 1571. 1925.

5) BENEDETTI-PICHLER, Die Fortschritte der Mikrochemie in den Jahren 1915 bis 1926. Wien. 1927. Vgl., TUNMANN-ROSENTHALER, Pflanzenmikrochemie. 2. Aufl. Berlin. 1931.

6) HELLER, K. und KRUMHOLZ, P., Beiträge zur systematischen Tüpfelanalyse. Mikrochemie. Bd. 7, S. 213. 1929.

Als Nachteil dieser Reaktion bei der kolorimetrischen Analyse wurde angeführt, dass die Ausfällung von Eisen zusammen mit Aluminium erfolgt. In der Tat bringen anorganische Eisenverbindungen ähnlichen Niederschlag hervor wie Aluminium. Es lässt sich jedoch durch seine purpurne Färbung von diesem leicht unterscheiden. Überdies kommt die Eisenreaktion in der Phytomikrochemie nur selten vor, weil, wie MOLISH[1] gezeigt hat, fast alles Eisen in Pflanzenzellen in maskierter Form vorhanden ist und sich mit mikrochemischer Reaktion nicht direkt nachweisen lässt. Selbst wenn Pflanzenmaterial, das sich durch locker gebundene Eisenaufspeicherung auszeichnet, wie z. B. *Trapa natans*, deren Fruchtschale bei der Blutlaugensalzprobe intensitive Blaufärbung zeigt, mit dem Reagens behandelt wurde, trat keine störende Reaktion auf. Daher konnten wir mit Alizarin-S-Reagens[2] das Vorhandensein von Aluminium im Pflanzenmaterial mit befriedegendem Ergebnis nachweisen.

Zum mikrochemischen Nachweis von Aluminium in Pflanzenzellen wurde ein Schnitt des Blatts auf den Objektträger gebracht und ein Tropfen des angegebenen Reagens hinzugefügt. Bald nach der Behandlung bemerkt man charakteristischen Aluminium-Lack auf dem Schnitt und am Schnittrande. Die Reaktion lässt sich daher zum lokalen Nachweis von Aluminium in Pflanzenzellen nicht verwenden, jedoch ist sie sehr empfindlich[3] und eignet sich für lebendes ebenso wie getrocknetes Material. Im vorliegenden Versuche wurden die meisten Prüfungen der Bequemlichkeit halber mit Herbarmaterial ausgeführt.

Obwohl man mit der phytomikrochemischen Reaktion das Vorhandensein der zu untersuchenden Substanz schneller und einfacher erfassen kann, so lässt sich damit doch die quantitative Menge nicht schätzen, wenn nicht die quantitative Makroanalyse mit ausgeführt wird. Man muss daher bei

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[1] MOLISCH, H., Mikrochemie der Pflanze. 3. Aufl. Jena. S. 41. 1923.

[2] Die Darstellung des angegebenen Reagens erfolgt nach STOCK folgendermassen: Man löst 100 g NH_4NO_3 und 4 g Alizarinsulfosaures Na (Kahlbaum), jedes für sich, in wenig Wasser, vereinigt sie zu einer unfiltrierten Lösung, verdünnt sie auf etwa 350 ccm, setzt 50 ccm Eisessig und unter kräftigem Umschwenken langsam 50 ccm 2-N NH_3-Lösung hinzu. Die Lösung wird auf 500 ccm aufgefüllt und nach 24 St. filtriert. Die Lösung wird vor dem Gebrauch durch Filtrierpapier gegossen, wodurch sie leicht völlig zu klären ist.

[3] Zur Bestimmung der Empfindlichkeitsgrenze des Reagens wurde ein Tropfen der Aluminiumsulfat-Lösung, die etwa 10 cmm Flüssigkeit enthielt, auf den Objektträger gebracht und nach dessen Verdunstung das Reagens hinzugefügt, damit sich roter Aluminium-Lack bildete. Die äusserste Verdünnung, bei der noch deutliche Reaktion eintrat, war eine 0.001 prozentige Lösung. Die Empfindlichkeitgrenze des Reagens beträgt also etwa 0.01 γ Aluminium in 10 cmm

der Makroanalyse immer erklären, inwieweit das gewonnene qualitative Ergebnis der Mikrochemie quantitativ zuverlässig ist. Nun wurde versucht, die am Schnitt mikrochemisch nachgeprüften Ergebnisse mit denen der Makroanalyse zu vergleichen. Dasselbe Blattstück, desen Schnitt zum mikrochemischen Nachweis des Aluminiums benutzt war, wurde daher makrochemisch analysiert. Dabei wurde das Material zunächst in einem Platintiegel verascht, dann nach Wägung die Reinasche mit Zusatz von Kaliumbisulfat geschmolzen und mit destilliertem Wasser angefeuchtet. Der Aluminiumgehalt der Asche wurde nach der Methode von STOCK kolorimetrisch mit dem Eisen mitbestimmt, dann der Eisengehalt mit Rhodan besonders und dieser abgezogen. Folgende Tabelle bringt einige Ergebnisse dieser Parallelversuche.

TABELLE I.

Name der Familie	Name der Pflanzen	Ausfall der Reaktion	Al (in der Asche.	Fe Proz.)	Asche (in Proz. d. Trocken-subst.)
Gramineae	*Miscanthus sinensis*	—	0.2	0.1	8.6
Fagaceae	*Quercus Myrsinaefolia*	—	0.6	Spur	8.7
Ulmaceae	*Ulmus parvifolia*	—	0.6	0.1	26.2
Urticaceae	*Boehmeria nivea*	—	0.3	Spur	20.8
	Debregeasia edulis	—	Spur	Spur	24.9
Calycanthaceae	*Chimonanthus praecox* var. *typicus*	—	0.5	Spur	27.2
Leguminosae	*Indigofera pseudotinctoria*	—	Spur	Spur	17.8
Theaceae	*Camellia japonica* var. *hortensis*	++	2.6	Spur	6.4
	C. Sasanqua	++	4.4	Spur	5.9
	Eurya japonica	++	4.9	0.2	6.5
	E. ochnacea	+	0.6	0.1	6.3
	Stewartia pseudocamellia	++	3.6	0.2	8.9
	Ternstroemia japonica	—	0.1	Spur	7.1
	Thea sinensis	++	2.7	Spur	8.8
Umbelliferae	*Peucedanum japonicum*	—	Spur	Spur	17.4

Name der Familie	Name der Pflanzen	Ausfall der Reaktion	Al (in der Asche. Proz.)	Fe (in der Asche. Proz.)	Asche (in Proz. d. Trockensubst.)
Diapensiaceae	*Diapensia lapponica* var. *asiatica*	╫	16.3	0.4	4.3
	Shortia soldanelloides var. *genuina* f.	╫	24.0	Spur	6.6
Ericaceae	*Leucothoe Grayana* var. *Tschonoskii*	+	1.4	Spur	6.4
Symplocaceae	*Symplocos crataegoides*	╫	13.7	Spur	10.6
	S. lucida	╫	22.7	0.1	10.7
	S. myrtacea	╫	27.2	0.4	13.0
	S. neriifolia	╫	25.9	Spur	9.7
	S. prunifolia	╫	19.2	Spur	9.9
	S. theophrastaefolia	╫	26.6	Spur	15.8
Rubiaceae	*Damnacanthus indicus* var. *major*	╫	6.7	0.2	7.2
	Oldenlandia hirsuta	++	4.0	Spur	8.9
Lycopodiaceae	*Lycopodium clavatum*	++	15.7	Spur	5.1
	L. complanatum var. *anceps*	╫	19.9	0.1	5.4
	L. sitchense var. *nikoense*	++	8.6	Spur	6.0
Isoetaceae	*Isoetes japonica*	—	0.1	0.1	22.9
Polytrichaceae	*Polytrichum juniperinum*	++	11.3	1.7	4.9

Wie aus der Tabelle zu erkennen ist, stimmen die Ergebnisse der mikrochemischen Prüfung im grossen und ganzen sehr gut mit denen der Makroanalyse überein, wenn der Aluminiumgehalt des Pflanzenmaterials ungefähr ein Prozent in der Asche überschritt. Geringere Mengen Aluminium als dies kann man nicht immer mittels dieser mikrochemischen Methode auf einem Blattschnitt nachweisen. Wenn man positive mikrochemische Reaktion erhält, so ist anzunehmen, dass das Material wenigstens ein Prozent Aluminium in der Asche enthält. Die Ergebnisse der Parallelanalyse zeigen ferner, dass die mikrochemisch gewonnenen Resultate fast immer den quantitativen Mengen des Aluminiums gut entsprechen, und zwar zeigt eine geringe Lackmasse unter dem Mikroskop nur etwa ein Prozent Aluminiumgehalt, während man aus einer grossen Masse den höchsten Wert erwarten kann, der wenigstens zehn Prozent Aluminium in

der Asche beträgt. Wir können daher durch die mikrochemische Prüfung mit diesem Reagens ein gutes Bild für den Aluminiumgehalt in den verschiedenen Pflanzen gewinnen, soweit es in ziemlich grosser Menge vorhanden ist. Im Anschluss an den Aluminiumgehalt haben wir uns davon überzeugt, dass in Blättern viel weniger Eisen als Aluminium aufgespeichert ist, das in den bestimmten Pflanzen in bemerkbarer Menge vorhanden ist.

II. Die Verbreitung von Aluminium im Pflanzenreiche.

Die Verbreitung von Aluminium innerhalb verschiedener Familien ist systematisch noch wenig untersucht worden. KRATZMANN[1] verdanken wir die durch mikrochemisches Verfahren gewonnene Erkenntnis, dass Aluminium im Pflanzenreiche weit verbreitet ist. Er gelangte weiter zu dem Schlusse, dass das Vorkommen von Aluminium *nicht* im Zusammenhang mit der systematischen Stellung der Pflanzen steht. Dieser Schluss gründet sich aber auf die von ihm geprüften 130 Pflanzen und etwa 80 Pflanzen, deren Aluminiumgehalt durch Aschenanalyse von WOLFF[2] gefunden worden war. In diesen ihren Versuchen wurden natürlich verschiedene Pflanzenteile zur Prüfung benutzt. Da der Aluminiumgehalt einerseits je nach den Organen verschieden und andererseits die Aschenanalyse die geringste Menge nachzuweisen vermag, so kann man aus diesen Ergebnissen nicht ohne weiteres die Beziehung der Aluminiumanhäufung zur systematischen Stellung der Pflanzen feststellen. Hier ist nun versucht worden, um ein klares Bild über die Verbreitung des Aluminiums im Pflanzenreiche zu erhalten, nur ein und dasselbe Organ bei den vergleichenden Versuchen zu benutzen, und zwar nur Schnitte von Blättern. Wie oben bemerkt, kann man mit dem Alizarin-S-Reagens den Aluminiumgehalt von etwas über einem Prozent in der Asche sicher nachweisen. Die positive Reaktion zeigt daher bereits eine hohe Anhäufung von Aluminium, so dass man solche Pflanzen geradezu als Aluminium-Pflanzen ansprechen kann. Mit diesem Reagens untersuchten wir eine grosse Anzahl von Pflanzen,[3] die zu verschiedenen Familien gehören und auch an ökologisch uneinheitlichen

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1) KRATZMANN, E., l. c.

2) WOLFF, E., zitiert in KRATZMANN, S. 318.

3) Das Material stammt zum grössten Teil aus den Sammlungen im botanischen Laboratorium auf Berg Hakkoda, es wurde aber auch Herbarmaterial unseres Instituts sowie frisches Material benutzt, das auf dem oben genannten Berg und im botanischen Garten in Sendai gesammelt worden war. Ich spreche hier Herrn Dr. HORIKAWA in Hiroshima meinen besten Dank aus, der mir viel Herbarmaterial, besonders von Kryptogamen zur Verfügung gestellt hat. (Y. Y.).

Orten[1] gewachsen waren, auf Aluminium. Die 808 geprüften Pflanzen umfassen 674 Phanerogamen, die zu 121 Familien gehören, und 134 Kryptogamen, einschliesslich 28 Bryophyten und 2 Flechten. Um einen Überblick über die Verteilung von Aluminium im Pflanzenreiche zu geben, werden wir sie zunächst in folgender Tabelle zusammenfassen:

TABELLE II.

	Phanerogamae	Cryptogamae				Gesamtzahl
		Pteridophyta		Bryophyta	Lichenes	
		Filicales	Lycopodiales, Equisetales, etc.			
Geprüfte Pflanzen	674(121)*	83(10)	21(5)	28	2	808
Aluminium-Pflanzen	42(15)	13(5)	7(2)	15	0	77

* Die Ziffern in Klammern zeigen die Anzahl von Familien.

Wir haben in etwa zehn Prozent der geprüften Pflanzen deutliche Aluminiumanhäufung gefunden, in Kryptogamen viel häufiger als in Phanerogamen. Auffallend ist, dass sich die 62 geprüften Aluminium-Pflanzen, ausschliesslich Bryophyten, in 22 Familien finden und unter diesen nur in 6 Familien massenhaft.

Die uneinheitliche Verteilung des Aluminiums in den verschiedenen Familien und Gattungen lässt sich aus der folgenden Tabelle III noch deutlicher erkennen. Um Raum zu sparen, wurden in der Tabelle nur die Pflanzen angegeben, die sich mittels dieser Reaktion als positiv erwiesen haben. Die Familien wurden systematisch nach ENGLER geordnet. Die Ziffern in Klammern neben den Familiennamen zeigen die Anzahl der von uns geprüften Pflanzen, und die folgenden die, bei denen das Reagens positive Reaktion gegeben. Durch die Anzahl der links von den Namen stehenden + ist der mehr oder minder bedeutende Aluminiumgehalt ersichtlich gemacht, während ± den undeutlichen Gehalt zeigt. Der von uns aus Aschenanalysen gewonnene Aluminiumgehalt wurde auch in Prozenten beigefügt.

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[1] Vergl. dazu unsere vorhergehende Arbeit:
YOSHII, Y. und JIMBO, T., Untersuchungen über die osmotischen Werte bei Pflanzen auf dem Berg Hakkoda. Science Reports Tohoku Imp. Univ. 4th Ser. (Biol.) Vol. 6, p. 259. 1931.

TABELLE III.

		Potamogetonaceae (4) 1
±	*Potamogeton polygonifolius* Pourr.	
		Liliaceae (27) 1
±	*Aletris foliata* Franch.	
		Lardizabalaceae (2) 1
±	*Stauntonia hexaphylla* Decne.	
		Saxifragaceae (14) 4
+ +	*Hydrangea hirta* Sieb. et Zucc.	
+	*H. opuloides* Steud. var. *Otaksa* Dipp.	
±	*H. paniculata* Sieb.	
±	*H. scandens* Maxim.	
		Rutaceae (5) 1
±	*Skimmia japonica* Thunb.	
		Euphorbiaceae (15) 1
+	*Daphniphyllum humile* Maxim.	
		Aquifoliaceae (6) 1
±	*Ilex crenata* Thunb. var. *typica* Loes. f. *genuina* Loes.	
		Theaceae (13) 11
+ +	*Camellia japonica* L. var. *hortensis* Makino. (2.6%)	
+ +	*C. japonica* L. var. *spontanea* Makino.	
+ +	*C. reticulata* Lindl.	
+ +	*C. Sasanqua* Thunb. (4.4%)	
+ +	*Eurya emarginata* Makino.	
+ +	*E. japonica* Thunb. (4.9%)	
+	*E. ochnacea* Szysz (0.6%)	
+ +	*Stewartia monadelpha* Sieb. et Zucc.	
+ +	*S. pseudocamellia* Maxim (3.6%)	
+	*S. serrata* Maxim.	
+ +	*Thea sinensis* L. (2.7%)	
		Cornaceae (6) 1
±	*Cornus canadensis* L.	
		Diapensiaceae (4) 4
+ + +	*Diapensia lapponica* L. var. *asiatica* Herd. (16.3%)	
+ + +	*Shortia soldanelloides* Makino var. *genuina* Makino f. *typica* Makino. (24.0%)	
+ + +	*S. soldanelloides* Makino var. *magna* Makino.	
+ + +	*S. uniflora* Maxim.	

Ericaceae (27) 1

\+ *Leucothoe Grayana* Maxim. var. *Tschonoskii* Takeda. (1.4%)

Symplocaceae (7) 7

\+ + + *Symplocos crataegoides* Ham. (13.7%)
\+ + + *S. lancifolia* Sieb. et Zucc.
\+ + + *S. lucida* Sieb. et Zucc. (22.7%)
\+ + + *S. myrtacea* Sieb. et Zucc. (27.2%)
\+ + + *S. neriifolia* Sieb. et Zucc. (25.9%)
\+ + + *S. prunifolia* Sieb. et Zucc (19.2%)
\+ + + *S. theophrastaefolia* Sieb. et Zucc. (26.6%)

Oleaceae (11) 1

\+ *Osmanthus Aquifolium* Sieb. var. *japonicus* Makino.

Lentibulariaceae (2) 1

\+ *Utricularia japonica* Makino.

Rubiaceae (26) 6

\+ + *Damnacanthus indicus* Gaertn. f. var. *genuinus* Makino.
\+ + + *D. indicus* Gaertn. f. var. *major* Makino. (6.7%)
\+ + + *Lasianthus japonicus* Miq.
\+ + *Mitchella repens* L. var *undulata* Makino.
\+ *Oldenlandia diffusa* Roxb.
\+ + *O. hirsuta* L. f. (4.0%)

Marattiaceae (1) 1

\+ *Angiopteris evecta* Hoffm.

Ophioglossaceae (5) 1

\+ *Botrychium ternatum* Sw.

Cyatheaceae (3) 3

\+ *Alsophila Bongardiana* Mett.
\+ *A. latebrosa* Hook.
\+ *Cyathea spinulosa* Wall.

Polypodiaceae (61) 6

\+ *Asplenium incisum* Thunb.
\+ *Dryopteris Miqueliana* C. Chr
\+ + *Plagiogyria adnata* Bedd.
\+ + + *P. Matsumuraeana* Makino.
\+ *Polystichum Thunbergii* Koidz.
\+ *P. tripteron* Presl.

Gleicheniaceae (2) 2

\+ + *Gleichenia glauca* Hook.
\+ *G. linearis* Clarke.

Lycopodiaceae (10) 6

+ + +	*Lycopodium*	*cernuum* L.
+ +	*L.*	*clavatum* L. (15.7%)
+ + +	*L.*	*complanatum* L. var. *anceps* Milde. (19.9%)
+ +	*L.*	*inundatum* L.
+ +	*L.*	*obscurum* L.
+ +	*L.*	*sitchense* Rupr. var. *nikoense* Takeda. (8.6%)

Isoetaceae (2) 1

+ +	*Isoetes*	*asiatica* Makino.

Hepaticae (11) 5

+	*Bazzania*	*albicans* St.
+	*Scapania*	*gigantea* Horikawa.
+	*S.*	*hirosakiensis* Steph.
+	*S.*	*rotundifolia* Horikawa.
+ +	*S.*	*spinosa* St.

Musci (17) 10

+	*Leucobryum*	*scabrum* Lac.
+	*Mnium*	*punctatum* (L., Schreb.) Hedw.
+ +	*Pogonatum*	*contortum* (Menz.) Loesk.
+	*P.*	*grandifolium* Jaeg.
+ +	*P.*	*inflexum* Lindb
+	*P.*	*urnigerum* (L.)
+	*P.*	*spinulosum* Mitt.
+ +	*Polytrichum*	*juniperinum* Willd. (11.3%)
+ +	*P.*	*sphaerothecium* Besch.
+	*Trachypus*	*bicolor* R. et H.

Aus der Tabelle kann man zunächst erkennen, dass das Aluminium auf bestimmte Familien beschränkt zu sein scheint, wenn es auch in ganz kleinen Mengen, die zwar nicht mittels dieses phytochemischen Verfahrens, wohl aber mit der Aschenanalyse nachweisbar sind, weit verbreitet sein mag. Wir möchten den Aluminiumgehalt in einigen Familien als eine ihrer charakteristischen Eigenschaften betrachten, weil ihre Vertreter alle oder fast alle eine grosse Menge von Aluminium in ihren Blättern aufspeichern. Wir konnten die bekannte Tatsache, dass die meisten *Lycopodiaceae*, Baumfarne und *Symplocaceae* reichlich Aluminium enthalten, auch bei unseren Pflanzen bestätigen. In der Tat zeichnen sich alle von uns geprüften *Symplocos*- Arten durch auffallend grosse Mengen von Aluminium aus, das bei den meisten Pflanzen über 25% (entspricht etwa 50% Al_2O_3)

in der Asche beträgt. Während die 3 geprüften Baumfarne (*Cyatheaceae*) ebenfalls positive Reaktion zeigen, lässt sich deutliche Anhäufung von Aluminium in den meisten *Lycopodiaceae* (6 Arten unter 10) feststellen. Dazu können wir noch die Familie *Gleicheniaceae* hinzufügen, da 2 geprüfte Vertreter gleichfalls deutliche Aluminiumanhäufung zeigten. Noch auffallender ist, dass alle Pflanzen der *Diapensiaceae* eine kolossale Menge von Aluminium aufspeichern, ebenso wie *Symplocaceae*. Ein anderes Beispiel haben wir in der Familie der *Theaceae* gefunden, obwohl der Aluminiumgehalt nicht so reichlich ist wie der der oben angegebenen Familien, jedoch gehören die meisten (11 Arten unter insgesammt 13) zu den Aluminium-Pflanzen.

Die Verteilung des Aluminiums in beschränkten Gattungen lässt sich noch klarer zeigen. Selbstverständlich zeichnen sich alle geprüften Gattungen *Symplocos* in den *Symplocaceae* und *Diapensia* und *Shortia* in den *Diapensiaceae* dadurch aus, dass ihre Vertreter ausnahmslos zu den Aluminium-Pflanzen gehören. Das Gleiche gilt auch für Gattungen in den *Gleicheniaceae*, *Cyatheaceae* und *Marattiaceae*, obwohl von den letzten nur ein Vertreter zur Verfügung stand. Die Gattung *Lycopodium* bietet ein anderes Beispiel, obschon sich eine bemerkbare Menge von Aluminium bei zwei Arten und zwei Varianten nicht erkennen lässt, die systematisch zu einer verwandten Abteilung gehören. Auffallend ist die Verteilung in den Gattungen von *Theaceae*; denn während alle geprüften Pflanzen in den Gattungen *Camellia*, *Eurya*, *Stewartia* und *Thea* eine ziemlich grosse Menge von Aluminium aufspeichern, ist das Metall bei Vertretern von *Schima* und *Ternstroemia* nicht deutlich angehäuft. Hervorgehoben sei hier noch, dass Aluminium-Pflanzen unter den *Saxifragaceae* alle zur Gattung *Hydrangea* gehören. Ein ähnlich auffallendes Beispiel lässt sich bei den *Rubiaceae* erkennen. In dieser Familie haben wir bei 6 Arten unter 26 geprüften eine beträchtliche Menge von Aluminium gefunden, wobei 2 zur Gattung *Damnacanthus* und 2 zu *Oldenlandia* gehören, während die übrigen 2, jede für sich, nur der einzige Vertreter der Gattung *Lasianthus* und *Mitchella* sind. Die Aluminium-Pflanzen finden sich beschränkt auch in bestimmten Gattungen unter den *Polypodiaceae*. Also alle von uns geprüften *Plagiogyria*-Pflanzen erwiesen sich als Aluminium-Pflanzen, während andere artenreiche Gattungen nur eine einzige oder zwei Aluminium-Pflanzen enthalten, z. B. nur eine unter 15 geprüften *Dryopteris*- und 2 unter 9 *Polystichum*-Arten.

Hier muss nun unsere Ansicht, dass Aluminiumanhäufung als ein charakteristisches Merkmal einer Abteilung der Pflanzen aufzufassen ist,

berücksichtigt werden. Wenn diese unsere Ansicht richtig ist, so muss es viele Familien oder Gattungen geben, die keine Aluminium-Pflanzen enthalten. Das beweisen besonders die folgenden Familien, in denen es keinen Aluminium aufspeichernden Vertreter gibt, soweit unser Verfahren das mit Sicherheit nachweisen kann, wie *Cycadaceae* (1)*, *Ginkgoaceae* (1), *Taxaceae* (3), *Pinaceae* (16), *Typhaceae* (1), *Sparganiaceae* (1), *Scheuchzeriaceae* (1), *Hydrocharitaceae* (2), *Gramineae* (31), *Cyperaceae* (27), *Araceae* (1), *Commelinaceae* (1), *Juncaceae* (3), *Amaryllidaceae* (2), *Dioscoreaceae* (1), *Iridaceae* (4), *Orchidaceae* (12), *Piperaceae* (1), *Chloranthaceae* (2), *Salicaceae* (5), *Myricaceae* (1), *Juglandaceae* (1), *Betulaceae* (7), *Fagaceae* (9), *Ulmaceae* (3), *Moraceae* (11), *Urticaceae* (6), *Proteaceae* (1), *Santalaceae* (1), *Loranthaceae* (1), *Aristolochiaceae* (2), *Polygonaceae* (13), *Chenopodiaceae* (2), *Amarantaceae* (2), *Phytolaccaceae* (1), *Aizoaceae* (2), *Caryophyllaceae* (3), *Nymphaeaceae* (3), *Ceratophyllaceae* (1), *Ranunculaceae* (18), *Berberidaceae* (3), *Magnoliaceae* (4), *Calycanthaceae* (1), *Lauraceae* (6), *Papaveraceae* (1), *Capparidaceae* (1), *Cruciferae* (6), *Droseraceae* (1), *Crassulaceae* (1), *Pittosporaceae* (1), *Hamamelidaceae* (2), *Rosaceae* (25), *Leguminosae* (19), *Geraniaceae* (9), *Oxalidaceae* (1), *Simarubaceae* (1), *Buxaceae* (1), *Empetraceae* (1), *Celastraceae* (4), *Staphyleaceae* (1), *Aceraceae* (8), *Balsaminaceae* (2), *Rhamnaceae* (1), *Vitaceae* (2), *Elaeocarpaceae* (2), *Tiliaceae* (2), *Malvaceae* (1), *Sterculiaceae* (1), *Dilleniaceae* (2), *Guttiferae* (2), *Violaceae* (7), *Flacourtiaceae* (1), *Stachyuraceae* (1), *Thymelaeaceae* (4), *Elaeagnaceae* (2), *Lythraceae* (1), *Rhizophoraceae* (1), *Oenotheraceae* (7), *Halorrhagaceae* (2), *Araliaceae* (9), *Umbelliferae* (16), *Pirolaceae* (2), *Myrsinaceae* (4), *Primulaceae* (6), *Sapotaceae* (2), *Ebenaceae* (1), *Styracaceae* (1), *Loganiaceae* (1), *Gentianaceae* (7), *Apocynaceae* (2), *Asclepiadaceae* (1), *Convolvulaceae* (1), *Borraginaceae* (5), *Verbenaceae* (4), *Labiatae* (16), *Solanaceae* (1), *Scrophulariaceae* (11), *Bignoniaceae* (1), *Orobanchaceae* (1), *Plantaginaceae* (2), *Caprifoliaceae* (11), *Valerianaceae* (1), *Dipsacaceae* (1), *Cucurbitaceae* (2), *Campanulaceae* (5), *Compositae* (49), *Hymenophyllaceae* (4), *Schizaeaceae* (1), *Osmundaceae* (3), *Marsiliaceae* (1), *Salviniaceae* (2), *Equisetaceae* (4), *Selaginellaceae* (4), *Psilotaceae* (1), *Lichenes* (2). Anderseits zeigte in den folgenden Familien nur ein Vertreter, meistens freilich nur undeutlich, positive Aluminium-Reaktion: *Liliaceae* (27), *Rutaceae* (5), *Euphorbiaceae* (15), *Aquifoliaceae* (6), *Cornaceae* (6), *Oleaceae* (11), und *Ophioglossaceae* (5). Diese Tatsache, dass nur eine Pflanze, aber die anderen derselben Familie keine nachweisbare

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*) Die Ziffern in Klammern zeigen die Anzahl der von uns geprüften Pflanzen.

Menge von Aluminium enthielten, bestärkt uns noch mehr in unserer Ansicht, aber stützt nicht die Behauptung von KRATZMANN[1], dass keine Beziehung zwischen Aluminium und systematischer Verwandtschaft bestehe. Anderseits scheint das Verhalten einiger Familien dem zu widersprechen, weil in *Rubiaceae*, *Saxifragaceae* und *Polypodiaceae* einige Aluminium-Pflanzen zerstreut auftreten. Betrachtet man dies aber genauer, so bemerkt man, dass die meisten Aluminium-Pflanzen doch auf einige Gattungen beschränkt anftreten, wie schon oben erwähnt. Ähnliches gilt auch für die Bryophyten, indem alle 5 *Pogonatum*- und die diesen verwandten 2 *Polytrichum*-Arten in Musci und 4 *Scapania*-Arten in Hepaticae als Aluminium-Pflanzen sich erweisen, während andere nur zerstreut in verschiedenen Gattungen gefunden wurden. Von anderen Familien, *Isoetaceae*, *Lardizabalaceae* und *Lentibulariaceae*, die auch Aluminium-Pflanzen enthalten, können wir derzeit noch nicht sicher sagen, ob sie zu den Aluminium-Familien gehören oder nicht, weil die Zahl der geprüften Vertreter für solche Entscheidung nicht genügt.

Die Anzahl der Pflanzen, die mit dem angegebenen Reagens positive Reaktion zeigen, wird mit der Versuchszahl zunehmen, jedoch sind wir, insoweit eben unsere Prüfung reicht, zur Überzeugung gelangt, dass die Aluminium-Pflanzen hauptsächlich auf bestimmte Familien oder Gattungen beschränkt auftreten, mögen auch individuelle Schwankungen vorkommen.

Überblicken wir nun unsere Ergebnisse, so schliessen wir uns an MOLISCH[2] an, der hinsichtlich der weiten Verbreitung des Inulins in der Familie der Kompositen behauptet hat, dass es zu den charakteristischen Eigenschaften dieser Abteilung gerechnet werden müsse, wenn es auch noch in anderen, damit nicht verwandten Familien auftritt. So dürfen wir mit gutem Rechte den Aluminiumgehalt in einigen bestimmten Familien oder Gattungen als eine charakteristische Eigenschaft von ihnen betrachten, wenn auch Aluminium noch in anderen Abteilungen in kleinen Mengen oder zufällig auftritt.

ZUSAMMENFASSUNG.

1) Zum mikrochemischen Nachweis kleiner Mengen von Aluminium in pflanzlichen Geweben ist Alizarin-S mit gutem Erfolg anwendbar.

2) Die Parallelversuche mittels Aschenanalyse zeigen, dass ein Aluminiumgehalt der Blätter, der etwas grösser als ein Prozent in der Asche ist,

[1] KRATZMANN, E., l. c. S. 334.
[2] MOLISCH, H., l. c. S. 9.

mit dem angegebenen Reagens auf einem Blattschnitt phytomikrochemisch gut erfassbar ist.

3) Mit dem Reagens untersuchten wir eine grosse Anzahl von Pflanzen (674 Phanerogamen und 134 Kryptogamen), die zu verschiedenen Familien gehören und an ökologisch uneinheitlichen Orten gewachsen waren.

4) Obwohl Aluminium im Pflanzenreiche in kleinen Mengen weit verbreitet vorkommt, findet es sich doch in grossen Mengen nur auf bestimmte Pflanzen beschränkt.

5) Es lassen sich Beziehungen zwischen Aluminiumgehalt und systematischer Verwandtschaft der Pflanzen sicher feststellen.

6) Alle oder fast alle Pflanzen in den folgenden Familien (unter 136 geprüften Familien) zeichnen sich durch deutliche Aluminiumanhäufung in ihren Blättern aus: *Symplocaceae*, *Diapensiaceae*, *Theaceae*, *Cyatheaceae*, *Gleicheniaceae* und *Lycopodiaceae*.

7) Bei den Pflanzen folgender Gattungen konnten wir eine beträchtliche Menge Aluminium finden: *Hydrangea* (*Saxifragac.*), *Camellia*, *Eurya*, *Stewartia*, *Thea* (*Theac.*), *Diapensia*, *Shortia* (*Diapensiac.*), *Damnacanthus*, *Oldenlandia*, *Lasianthus*, *Mitchella* (*Rubiac.*), *Symplocos* (*Symplocac.*), *Angiopteris* (*Marattiac.*), *Alsophila*, *Cyathea* (*Cyatheac.*), *Plagiogyria* (*Polypodiac.*), *Gleichenia* (*Gleicheniac.*), *Lycopodium* (meistens) (*Lycopodiac.*), *Scapania* (*Hepaticae*), und *Pogonatum* (*Musci*).

Berichtigung.

In dem Aufsatz von YOSHII und HAYASI: „Botanische Studien subalpiner Moore... " (Contributions from the Mt. Hakkoda Bot. Laboratory No. 9) in Bd. 6, Nr. 2, 1931, Seite 340, Zeile 16–17 ist Richtigstellung eines Druckfehler unterblieben. Es soll richtig heissen: „Die Moore sind auf saurem, *nährstoffarmem* Aschendoden aus oligotrophen Pflanzen entstanden, wie...."

Toxic Action of the Stomach Extracts of the Starfishes on the Heart of the Oyster.[1]

BY

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(With 16 text-figures)

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INTRODUCTION.

It is well known fact that the starfish attacks the Pelecypodal and the Gastropodal molluscs, excreting some poisonous substance capable of paralysing or even to killing on the prey.

VAN DER HYDE (1922) demonstrated experimentally the presence of the toxic substance in the stomach extract of the starfish, using the isolated heart of *Pecten* and the gastrocnemius of frog. He, however, did not mention which species of the starfish was used, and states: "Does the stomach of the starfish secrete a substance which is poisonous to the muscles or in general, to the contractil tissues of the prey? In that way the secretion of the stomach would have a double function: 1. to kill the prey or at least to abolish the tonus of the adductor muscle, 2 to dissolve the tissues more or less completely in order to make them fit to enter into the stomach or the radial sacs."

We have undertaken to test his suggestion and to get more light on the nature of the toxin. Among the common starfishes, we have selected the species which have the most intense toxin and seemed suitable for farther investigations. Similar tests were made using the extracts of the alimentary tracts of the representative forms of the other classes of Echinodermata, as well as of the extract prepared from several tissues of the oyster.

We wish to express here our sincere thanks to Prof. S. HATAI, the director of the station, who gave us encouragement and suggestions throughout the investigation.

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[1] Contributions from the Marine Biological Station, Asamushi, Aomori-Ken. No. 79.

EXPERIMENTS.

The materials were prepared as follows. The tissues were removed from the animal body and washed in sea water, the excess sea water was absorbed by a filter paper and then weighed, ground in a mortar to paste and mixed well with sea water 15 times its own weight, and left for a while before use. The oyster heart used for testing the toxity of the extracts was cautiously isolated from *Ostrea circumpicta*, after the method described by TAKATSUKI (1927). The species name of the starfishes used, was identified by the descriptions given by UCHIDA (1928).

To examine the effect of the extracts on the heart, the sea-water was quickly replaced by a beaker containing a known amount of the extract, and the reactions produced were recorded by means of the usual kymographic method.

RESULTS.

I. The effect of the stomach extracts of the starfishes.

By the above methods, the effect of the extracts of five species of starfishes on the heart of oyster, gave the following results.

1) *Asterias rollestoni*. (Fig. 1.) On immersing the heart in the extract,

Fig. 1. Effect of the extract of the stomach of *Asterias rollestoni*. Time in 5 seconds.

the heart ceases pulsation almost instantly producing a tetanic contraction, which however gradually relaxes or shows at the most only one or two paroxysmical pulsations. The heart, thus treated for a few minutes, can not restore activity even when returned to normal sea water.

2) *Patiria pectinifera*. (Fig. 2) The heart when immersed into the extract of *Asterina*, shows no such fatal toxic effect. The heart shows a slightly increased number of pulsations in the course of six minutes, which however recovers when returned to sea water, with a tendency of slight

Fig 2. Effect of the extract of the stomach of *Patina pectinifera.* Time in 5 seconds.

acceleration in its pulsation.

3) *Certonardoa semiregularis.* (Fig. 3) The result is similar to that of *Asterina pectinifera,* though its toxity appears to be less.

4) *Astropecten scoparius.* (Fig. 4) The heart ceases pulsation in a paralized and contracted state, and does not recover in sea water. Thus

Fig. 3. Effect of the extract of the stomach of *Certonardoa semiregularis.* Time in minutes.

Fig. 4. Effect of the extract of the stomach of *Asteropecten scoparius.*

the effect resembles that of *Asterias rollestoni,* though its toxity appears weaker than that shown by *Asterias.*

5) *Aphelasterias japonica.* (Fig. 5) The heart beats normally in this

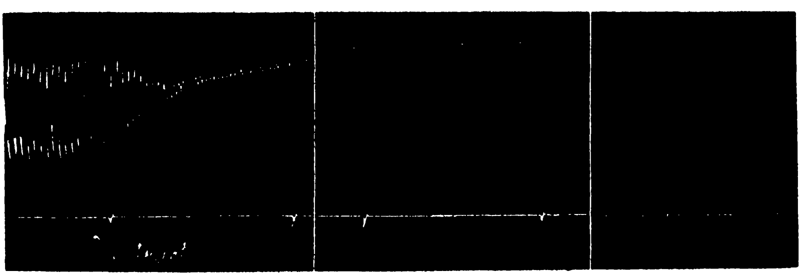


Fig. 5. Effect of the extract of the stomach of *Aphelasterias japonica.* Time in minutes.

extract for several seconds, but suddenly diminishes strikingly and after

about five minutes it ceases completely in a contracted state. The heart does not recover in the sea water.

II. Experiments on the Echinoidea and Holothuroidea.

The experiments were performed with the extracts of the intestinal tracts, and the following results were obtained.

1) *Strongylocentrotus sp.* (Fig. 6) The heart beating is remarkably accelerated in the extract, the frequency is increased very much, and no other toxic signs are observed.

Fig. 6. Effect of the extract of the intestine of *Strongylocentrotus sp.* Time in minutes.

2) Heart urchin. (Fig. 7) Practically no change is observed in the extract.

3) *Sticopus japonicus.* (Fig. 8) The frequency is increased and the

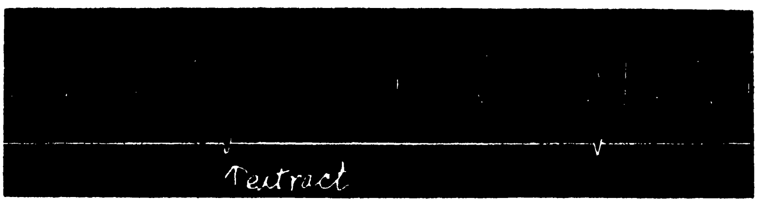


Fig. 7. Effect of the extract of the intestine of heart urchin. Time in minutes.

amplitude is nearly normal. On returning to sea water, the normal pulsations, if not slightly stronger, are recorded.

4) *Cucumaria* (Fig. 9) and *Caudina.* (Fig. 10) The effects are practically identical to that shown by *Sticopus japonicus.*

III. Distribution of toxic substance in *Asterias rollestoni.*

We have examined several other tissues than the stomach of *Asterias rollestoni*, and obtained the following results.

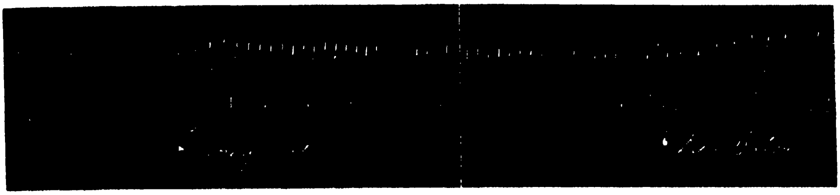


Fig. 8. Effect of the extract of the intestine of *Sticopus japonicus*.

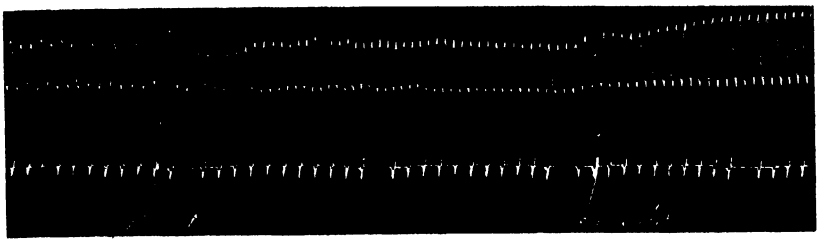


Fig. 9. Effect of the extract of the intestine of *Cucumaria sp.* Time in 5 seconds.

Fig. 10. Effect of the extract of the intestine of *Caudina chilensis*. Time in minutes.

1) Pyloric caeca. (Fig. 11) The heart immersed in the extract of pyloric **caeca** exhibits only a slight decrease in the amplitude.

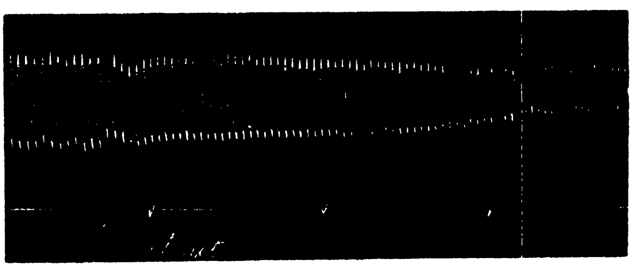


Fig. 11. Effect of the extract of the pyloric caeca of *Asterias rollestoni*. Time in minutes.

2) Tube-feet. (Fig. 12) The effect in the tube-feet appears within one minute, the amplitude gradually diminishes to half of the normal, though

Fig. 12. Effect of the extract of the tube-feet of *Asterias rollestoni*. Time in minutes.

the pulsation continues in such a state. When replaced in sea water the heart restores normal pulsation very slowly.

IV. Effects of the extracts of some tissues of oyster.

1) Digestive diverticula with the stomach wall. (Fig. 13) The digestive diverticula and stomach were ground together, and the extract was made

Fig. 13. Effect of the extract of the digestive diverticula of the oyster. Time in 5 seconds.

as usual. The heart pulsation in this extract shows a remarkable increase in the frequency, though at the same time the amplitude is considerably diminished. On returning to the sea water, the heart restores normal

Fig. 14. Effect of the extract of the adductor muscle of the oyster. Time in minutes.

pulsation with a slight tendency of increased frequencies.

2) Muscle (adductor). (Fig. 14) In the extract of the adductor muscle the heart beats somewhat rapidly with a much diminished amplitude.

3) Heart. (Fig. 15) The heart was extracted with sea water 20 times its own weight. The heart beats in a contracted state within the first half minute, but when, however, it is replaced to the sea water it gradually

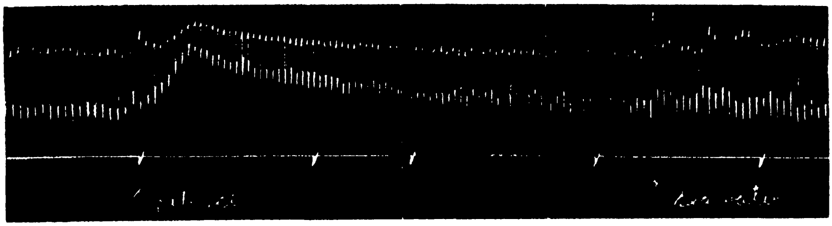
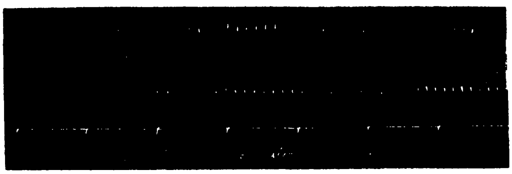


Fig. 15. Effect of the extract of the heart of the oyster. Time in minutes.



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a

3.0 cc

b

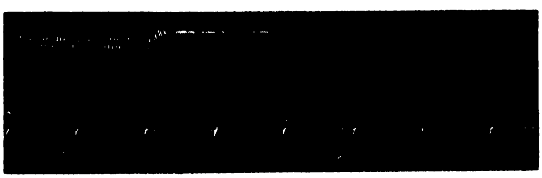

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Fig. 16. Effect of the extract of the stomach of *Asterias rollestoni*, in increasing the concentration of the extract by adding 1 cc., 1 cc., 3 cc., 5 cc., 10 cc. and finally 30 c of the extract to 100 cc. of sea water. Time in minutes.

regains the normal state and the pulsation becomes somewhat accelerated, especially in its amplitude.

V. The concentration of the extract at which the toxic reaction appears on the heart of oyster. (Fig. 16 a, b, c.).

The extract is prepared as usual, diluting the tissue paste of the stomach of *Asterias rollestoni* with the sea water of 15 times its own weight.

The reaction of the heart treated with the gradually incryased concentration of the extract, was shown in the following manner. The heart which was pulsating normally in the sea water, was replaced into the mixture of 1 cc. of the extract and 100 cc. of sea water, and the reaction in this medium was recorded; and after two minutes the heart was replaced into sea water for another two minutes. The heart thus recovered was again immersed into a mixture consisting of 2 cc. of the extract and of 100 cc. of sea water. The process was repeated till the amount of the extract was increased from 1 to 2, 5, 10, 20 and finally to a 50 cc. mixture with 100 cc. of sea water.

The results of the observations were as follows. In the concentration of the extract of 5 cc. in 100 cc. of sea water, the heart pulsates with less than half of the normal amplitude, and will not, within two minutes, restore normal pulsation when returned to sea water. When immersed in the mixture containing 20 cc. of extract in 100 cc. of sea water, the heart pulsation became very faint and small, and finally in the mixture of 50 cc. of the extract and of 100 cc. of sea water, the heart became paralysed and ceased pulsation in a contracted state.

CONCLUSION.

The present investigation shows that the poisonous strength of the stomach extracts differs widely according to the species of starfishes. The toxity is strongest in the extract prepared from the stomach of *Asterias rollestoni* and almost equally strong in that of *Asteropecten scoparius*. *Aphelasterias japonicus* occupies the middle while it is negative in those obtained from *Patiria pectinifera* and *Certonardoa semireguraris*.

In the extracts prepared from the digestive tracts of Echinodermata belonging to the classes Echinoidea and Holothuroidea, appearently no comparative poisonous substances are found.

The toxic reaction of the extract prepared from the stomach of *Asterias rollestoni* appears only when the concentration reaches 5 cc. of the extract in 100 cc. of sea water. The mixture of 20 cc. of the extract to 100 cc. of

sea water produces a decisive effect to the heart, and in the mixture of 50 cc. of extract in 100 cc. of sea water the heart is almost exhausted and no pulsation occurs.

The result obtained from the extract of the tube-feet shows that the poisonous substance is found in a slight amount, which toxity corresponds to about one tenth of that shown by the stomach extract.

In the extract prepared from a mixture of the digestive diverticula and stomach of oyster, the heart is excited greatly, but on the contrary in the adductor muscle extract, the pulsation is depressed remarkably. And in the extract prepared from the hearts (the tissue paste was diluted with sea water twenty times its own weight), the heart shows a slightly altered reaction at the beginning, but gradually restored to the normal state. When it was returned to the sea water, the heart pulsation became more vigorous.

Why the toxity of the stomach extract in one species is stronger than in the others may be correlated with the difference of the prey on which they live. *Asterias rollestoni*, the most poisonous species, attacks the large Pelecypodal and Gastropodal molluscs while the least poisonous species most probably attack the smaller and more delicate molluscs.

Asterias rollestoni, the most suitable species for the study of the starfish toxin, is found abundantly in the neighbourhood of the station and, in the near future we hope to report and discuss the nature of the toxin itself.

TABLE I.

Summarizing the results of the experiments.

Class	Species	Organ Extracted	Concent. of Ext.	Toxic React.	Remarks
ECHINOIDEA	1) *Strongylocentrotus sp.*	Intestinal tract	1:15	—	Fig. 6
	2) *Echinarachnius mirabilis*	,,	1:15		
	3) Bunbuku-chagama (Heart urchin)	,,	1:15	—	7
ASTEROIDEA	1) *Asterias rollestoni*	Stomach	1:15	⧻	1
		Tube-foot	1:15	+	12
		Pyloric caecum	1:15	— ?	11
	2) *Patiria pectinifera*	Stomach	1:15	+	2
	3) *Certonardoa semiregularis*	,,	1:15	— ?	3

4) *Asteropecten scoparius*	,,	1:15	++	4
5) *Aphelasterias japonica*	,,	1:15	++	5
HOLOTHUROIDEA 1) *Stichopus japonicus*	Intestinal tract	1:15	—	8
2) *Cucumaria* sp.	,,	1:15	—	9
3) *Caudina chilensis*	,,	1:15	—	10
Ostrea circumpicta	Digest. div.	1:15	—	13
	Adduct. muscle	1:15	—	14
	Heart	1:20	—	15

SUMMARY.

1. Observations were made on the effects of the extracts of the alimentary tracts of several forms of Echinodermata on the heart of the oyster.

2. Among the five species of starfishes examined, the most powerful toxin is found in the stomach extract of *Asterias rollestoni.*

3. Among the extracts prepared from the other tissues of *Asterias rollestoni,* that of the tube-feet gave slight toxic action.

4. The concentration of the extract of *Asterias rollestoni* at which the toxic action appears on the heart was determined.

5. The effects of the extracts prepared from the several organs including the heart tissue of the oyster were determined from the reactions shown by the heart of the same species,

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On the Early Localization and History of the So-Called Primordial Germ-Cells in the Chick Embryo. (Preliminary Report).

BY

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(With 35 text-figures)

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The diverse views, which have been put forward by various investigators, in regard to the origin of the primordial germ-cells in vertebrate animals, are grouped into three categories by McCOSH ('30), and into four by HEYS ('31). Nevertheless, it appears to me that the views put forward in the field of birds may be grouped naturally in two main divisions, as follows:

I. The germinal epithelium theory.

WALDEYER ('70) first put forward the idea that in the chick the germ-cells arise from the cells of the germinal epithelium. GATENBY ('24) also advocates this theory in the case of *Gallus bankiva*. In the case of the other classes of vertebrates, SEMPER ('75), BALFOUR ('77), MACLEOD ('81), HOFFMANN ('86), BÖHI ('04), ALLEN ('04, '23), GATENBY ('16), SIMKINS ('23, '24, '25, '28), HARGITT ('23, '25, '30 a, b, c), OBRESHKOVE ('24), PAPANICOLAOU ('25), SWEZY and EVANE ('30), and SIMKINS and ASANA ('30) may be named as supporters of the germinal epithelium theory. GATENBY ('23) states that it is indisputable that in the vertebrates below the mammals seasonal accessions of new germ-cells take place.

II. The theory of early segregation.

In the second division are grouped the views which show agreement in recognizing the early segregation of germ-cells. Since, however, there are many different conclusions concerning the origin, history and the ultimate fate of the so-called primordial germ-cells, these may be categorized again into two subdivisions: viz. a) those which maintain that, besides the primary germ-cells, which arise by early segregation, there are secondary germ-cells, which are derived from the germinal epithelium, and b) those which maintain that the definitive germ-cells arise only from the early segregated primordial germ-cells.

a) SEMON ('87), D'HOLLANDER ('04), and FIRKET ('14, '20) hold to the view that the primordial germ-cells in the fowl arise in the extra-embryonic regions and migrate into the gonads, but that many of them degenerate after their arrival in these, and most of the definitive germ-cells arise from the germinal epithelium. VON BERENBERG-GOSSLER ('12, '14) states also that in the chick the primordial germ-cells, — though he doubts the true nature of these cells, — may form the definitive germ-cells, but these may also come from the cells of the genital ridge. In the other field of vertebrates, FELIX ('06), DUSTIN ('07, '10), WINIWARTER and SAINMONT ('09), KUSCHAKEWITSCH ('10), ABRAMOWICZ ('13), STIEVE ('27), BUTCHER ('28, '29), and McCOSH ('30) recognize the secondary formation of germ-cells. BOUIN ('01) states that in *Rana* the secondary functional germ-cells arise not only from the peritoneum, but also from the mesenchyme, of the genital ridge.

b) NUSSBAUM ('80) first put forward the view that the definitive germ-cells are not in any case derived from the germinal epithelium, but are segregated in the extra-embryonic region in a much earlier stage of the embryo. HOFFMANN ('92), NUSSBAUM ('01), and RUBASCHKIN ('07) found, in bird embryos possessing 22 to 23 somites, typical germ-cells in the entoderm and splanchnic mesoderm lateral to the coelomic angle, and in embryos older than those they found also typical germ-cells which had passed into the germinal epithelium, but they could recognize no secondary origin of the germ-cells.

SWIFT ('14, '15, '16) asserted that in the chick the primordial germ-cells arise anterior and antero-lateral to the embryo in a specialized region of the germ-wall entoderm just at the margin of the area pellucida in the embryos from the primitive-streak to about 3-somite stage, that they are at first in the space between the entoderm and the ectoderm and then in the blood-vessels, and that they are at first carried by their own movement, but later by that of the blood, to all parts of the embryo and vascular area, where they remain generally distributed in this way until the embryo has about 20 somites. REAGAN ('16) states that the extra-regional origin of the germ-cells in the chick may be regarded as highly possible from the standpoint of the experimental proof, in which the germ-wall entoderm was extirpated in front of the primitive streak and no primitive germ-cells in the genital ridge resulted.

RICHARDS, HALPIEU and GOLDSMITH ('26) traced the ova back to the primordial germ-cells, and believe that no proliferation of germ-cells occurs in the germinal epithelium. GOLDSMITH ('28), corroborating the work of

SWIFT, states that the primordial germ-cells are of extra-embryonic origin and give rise to the definitive germ-plasm, and that no transformation of germinal epithelial cells into definitive germ-cells occurs.

In the case of the other classes of vertebrates, GOETTE ('75, '90), EIGENMANN ('91, '96), WHEELER ('99), BEARD ('00, '02), WOODS ('02), ALLEN ('06, '07 a, b, '09, '11 a, b), RUBASCHKIN ('08, '09, '12), JARVIS ('08), KING ('08), DODDS ('10), BACHMANN ('14), JORDAN ('17), OKKELBERG ('21), RICHARDS and THOMSON ('21), REINHARD ('21), BURNS ('25), HANN ('27), and WITSCHI ('29) are also supporters of the view which maintains the continuity between the primordial and definitive germ-cells. WOLF ('31) states with reference to *Platypoecilus maculatus* that in the male the primordial germ-cells are the sole source of the definitive elements, but that in the female this is not the case but that some of the cells of the germinal epithelium are transformed also into the ova.

TSCHASKIN ('10) states that in the bird embryo the germ-cells are first found in the somatic and splanchnic mesoderm, but he gives no information regarding the history of these cells. WOODGER ('25) states that in the fowl there is no doubt about the continuity of the primitive germ-cells in the genital ridge with those in the splanchnic mesoderm of earlier stages and with the large cells of the blood-stream in still earlier ones, but he gives us no information about the ultimate fate of the primitive germ-cells.

On a perusal of the papers concerning the birds, it appears that in the study of the development of the germ-cells three main questions arise. The first is what is the origin and early history of the so-called primordial germ-cells which are found in the forming genital ridge in the stage of about a 4-day incubation? The second is whether the so-called primordial germ-cells will degenerate or not. The third is whether the definitive germ-cells will develop also from the cells of the germinal epithelium. Of course all the solutions must be proved not only histologically but experimentally if possible.

Among many investigators who have traced the primordial germ-cells GOLDSMITH ('28) alone thoroughly studied the cells from the 12-hour embryo stage to that of the adult, his work, however, being much influenced by the observations of SWIFT. Both SWIFT and GOLDSMITH believe that the primordial germ-cells arise anteriorly and antero-laterally to the head fold during the primitive-streak stage, on the basis of identifying the germ-cells with the entodermal wander-cells of DANTSCHAKOFF ('08), which are said to bud off from the germ-wall entoderm anterior to the primitive streak. This view has been verified by REAGAN ('16).

The present study was undertaken in March, 1930, at the suggestion of Prof. Dr. E. NOMURA, to whom I am greatly indebted for his valuable criticisms and helpful advice and to whom I wish to express my heartfelt thanks for his most kind revising the proof of this manuscript. I began the research of the origin and early history of the so-called primordial germ-cells merely to determine the answer to the first question stated above, and I found these germ-cells not in the area anterior to the embryonic axis, but, as EIGENMANN ('91) states in the case in *Micrometrus*, in the embryonic axis itself. I have, of course, to study this problem also experimentally, but as my histological investigation needed more time than I had expected, and the time available being limited, owing to my having soon to be called up for military service, I am obliged to publish this paper as a preliminary report without accomplishing the experimental part.

MATERIALS AND METHODS.

As materials for the investigation, the eggs of thoroughbred white Leghorns were mainly used, because of the high rate of the fertilized eggs of this species. Sometimes the eggs of black Orpingtons were also used. Both kinds were bred in my own poultry yard, the adults having been kindly presented to me by Mr. J. TOMODA and Mr. T. MITSUI, members of the Central Poultry Association, to whom I herewith express my thanks.

In order to know when the eggs were laid, a number of trap-nests were used. The embryos of these eggs for the main part of my work were preserved at desired intervals of incubation from 10 hours to 4 days. Especially, the embryos younger than the one-somite stage were obtained at incubation intervals of one or two hours. Moreover, in addition to these stages, for the purpose of becoming familiar with the so-called primordial germ-cells in the developing gonad or genital ridge, I also studied embryos 5½ days, 5 days, and 4½ days old.

In general, special care was taken to determine the developing stages of the embryos. Those younger than the one-somite stage were distinguished according to the lengths of the primitive streak and notochord as well as the number of hours of incubation. In the case where the embryos were in the 1-somite to the 25-somite stages, the size of the embryo, the number of somites as well as the number of hours of incubation were referred to as the standards. In the stages where there were more than 25-somites, several morphologies of the embryo together with the number of hours of incubation were involved. Unusual embryos, either larger or smaller than

the normal, whenever met with, were not taken into account.

In the course of my tests I found the TOMODA Petroleum Incubator well-adapted for keeping a constant temperature, viz. 104° ± 1°F. This incubator was presented to me by Mr. K. CHIBA, a member of the Central Poultry Association, to whom I wish also to express my thanks.

All the embryos used as specimens were studied in serial paraffin cross-sections, 5 micra in thickness, and were sketched or photographed for reconstruction before cutting. Moreover, in order to prevent loss of free-cells especially those in blood-vessels, while staining was being carried on, immediately after the removal of the paraffin in xylol, each slide was put in a celloidin solution to make a thin film over the sections.

A few embryos were fixed with BENSLEY's acetic–osmic–bichromate mixture, and to them two methods of staining were applied, one being anilin–acid fuchsin and methyl green, the other BENSLEY's ('11) copper–chrom–hematoxylin. To some materials, after fixation with FLEMMING's fluid modified by MEVES, iron–hematoxylin stain was applied. These three methods were suitable for embryos older than the 15-somite stage, as SWIFT ('14) states, but were useless in the case of the younger embryos, the nucleus and cytoplasm being obscured by numerous blackened yolk spheres in those stages.

The materials were fixed by SWIFT's method,- with a mixture of equal parts of 5 percent trichlor acetic acid and 5 percent corrosive sublimate, and one part of them was stained with iron-hematoxylin and acid fuchsin, and the other with AUERBACH's fuchsin and methyl green. Some materials, after fixation with a mixture of equal parts of saturated picric acid and saturated corrosive sublimate, were stained with iron-hematoxylin and acid fuchsin, with DELAFIELD's hematoxylin and eosin, with AUERBACH's fuchsin and methyl green, or with picro-carmine. In the case of some materials, which were fixed with acetic sublimate, DELAFIELD's hematoxylin and eosin stain was used. Among these several methods, the iron-hematoxylin and acid fuchsin stain after the picro-sublimate fixation was the best for embryos younger than the 15-somite stage. For very young embryos, the cells of which are rich in yolk spheres after from a 10-hour to an 18-hour incubation, the picro-carmine stain was most effective.

Table I was prepared to show at a glance the number of embryos used as specimens together with their age and the methods used for fixation and staining.

TABLE I.

Fixation	Stain																							
Number of somites										2	3	4	6	8	10			12	14	15	16	18	20	23
Days of incubation													1							1½				
Hours of incubation		10	12	14	15	16	17	18	19	20	21	22	24	26	28	30	31	33	34	36	38	40	42	44
Saturated picric acid and saturated corrosive sublimate fixation	Iron-hematoxylin and acid fuchsin stain		1	1	1	2	1	6	3	2	2	2	3	2	1	1	2	1	2	2	2	2	2	1
	DELAFIELD's hematoxylin and eosin stain		1	1		1		1		1			1											
	AUERBACH's fuchsin and methyl green stain		1	1		1		1																
	Picro-carmine stain in bulk	4	2	2		3		2																
Trichlor acetic acid (5%) and sublimate (5%) fixation	Iron-hematoxylin and acid fuchsin stain		1			2		2					1	1	1					1	1		1	
	AUERBACH's fuchsin and methyl green stain		1										1							1				
Acetic sublimate fixation and DELAFIELD's hematoxylin and eosin stain			1			1		1		1			1		1					1			1	
MEVES' fixation and iron-hematoxylin stain						2		1				1	1	1	1				1	1	1	1	1	1
BENSLEY's acetic-osmic-bichromate fixation	Anilin-acid fuchsin and methyl green stain																							
	BENSLEY's copper-chrome-hematoxylin stain																							

26	27	29					36		38			41				Colour differentiation					
	2					2½	3		3½			4	4½	5	5½						
46	48	50	54	56	58	60	72	78	84	87	90	96	108	120	132	Cytoplasm	Nucleus	Nucleolus	Attraction sphere	Yolk sphere	Mito-chondria
2	2	1	1	1	1	2	2	1	2	1	1	2	1	1	1	light red	black	black	dark red	black	
																red	violet	violet	red	red	
																blue	(chromo-some red or green)	red	blue	blue	
																yellowish red	dark red	dark red	dark red	golden yellow	
1		1	1	1	1											light red	black	black	dark red	black	
	1						1					1				blue	(chromo-some red or green)	red	blue	blue	
						1	1					1			1	red	violet	violet	red	red	
1	4					1	1		1			1				light black	black	black	light black	black	black
	1					1	1		1			1			1	blue	red	red	blue	black	red
	1					1	2		3			3	1	1	1	light brown	black	black	brown	black	bluish black

IDENTIFICATION OF THE PRIMORDIAL GERM-CELLS.

It is very important to describe here the morphological characters of the so-called primordial germ-cells before entering on a description of my own observations.

The most prominent criteria for the identification of the germ-cells are their size and shape. The germ-cells are always round or oval in shape and measure 15 to 18 micra in diameter, and they are always larger than the somatic cells. They retain their size and shape, more or less, at least until the fourth or fifth day of incubation, even if the somatic cells become smaller as a result of their successive divisions. Sometimes these somatic cells may be also round or oval, though they are frequently stellate, flattened or elongated.

The germ-cell nucleus is always spherical and vesicular and appears to be larger, measuring about 9 micra, and clearer than the somatic-cell nucleus. It is always eccentric in position, being surrounded by cytoplasm, which is thinner on one side and thicker on the other.

The presence of a large attraction sphere, situated on the thicker side of the cytoplasm and attached to the large nucleus, is also a prominent feature by which the germ-cells may be distinguished from the somatic cells, which show no trace of such spheres. " Although my material ", states GOLDSMITH ('28), " does not show the attraction sphere as prominent as SWIFT figures it, it is nevertheless prominent enough for use ". As to my own material, in the early stages the attraction sphere was not sometimes so prominent, but in the older stages, from 3 to 5½ days, it was as prominent as SWIFT figures it.

Most frequently one plasmosome, but sometimes two are found within the germ-cell nucleus, but their size and position are quite indeterminable. It is stated by DODDS ('10) that in the germ-cells of teleostei two small plasmosomes are found quite far apart from each other close to the nuclear membrane.

Yolk material also is a characteristic constituent of the germ-cells. The latter contain sometimes more numerous or larger spheres than do the somatic cells, and in the later stages, even if the yolk contents decrease in amount, they still remain in the germ-cells long after they have disappeared from the somatic cells.

In the chick embryo, the mitochondria of the germ-cells resemble those of the somatic cells and do not seem to be a suitable characteristic to distinguish the former cells from the latter. This is one reason why I

preferred the iron-hematoxylin method, and gave up the staining method for mitochondria.

In conclusion, the only sure way to identify the germ-cells is to investigate all the characteristics in combination, viz. the large size and round shape of the cells, the large size and vesicular condition of the nucleus, the presence of the attraction sphere, and the quality and quantity of yolk contents.

PRIMORDIAL GERM-CELLS IN THE STAGES FROM THE PRIMITIVE-STREAK TO THE 10-SOMITE. (FROM 10 TO 30 HOURS INCUBATION).

The positions of the so-called primordial germ-cells are described in this paper, for convenience, in the order from the earliest stage to the more developed, though the actual tracing was in the reverse order, i. e. from the more developed stage to the earliest.

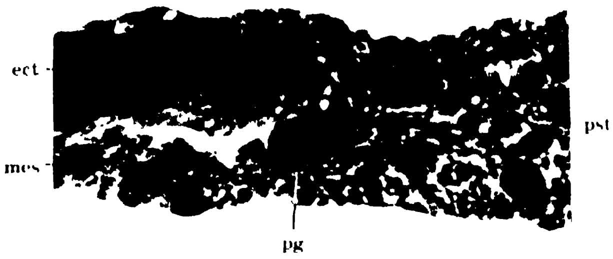

Fig. 1. Portion of the transverse section through 1–1 in Fig. 3. 10-hour incubation. Picro-sublimate fixation and picro-carmine stain. ×700 Photographed. *ect* ectoderm, *mes* meso-entoderm, *pg* primordial germ-cell, *pst* cell-mass of the primitive streak.

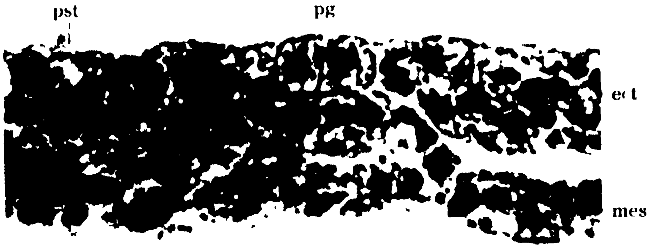

Fig. 2. Portion of the transverse section through 2–2 in Fig. 3. 10-hour incubation. Fixation and staining as before. ×700. Photographed. Abbreviations as before.

In the embryos after a 10-hour incubation, in which the primitive groove is not yet formed, the primordial germ-cells are found embedded in the meso-entoderm just lateral to the primitive streak (Fig. 1) as well as in the primitive streak itself (Fig. 2) and in the primitive plate. The distribution and the number of the germ-cells observed in one embryo of this stage are indicated in Fig. 3. Nearly the same arrangement can be seen even in embryos on a 12-hour incubation after the appearance of the primitive groove.

In the embryos in about the 14-hour stage, the primordial germ-cells are distributed mainly in the posterior half of the primitive streak, which is much elongated in this stage probably owing to the specially rapid lengthening of its anterior region (Fig. 4), and are found not only in the primitive streak and adjacent meso-entoderm, but even in the space between the ectoderm and meso-entoderm near the embryonic axis (Fig. 5). Thus some of the germ-cells become freed from the primitive tissues and pass into the segmentation cavity with the mesodermal cells. This movement ot the germ-cells, in my opinion, is probably not due to their active migration, but to mutual displacement between the primitive tissues passive in its nature. It is also worthy of note here that my photograph, shown in Fig. 5. is quite different from, in spite of its resemblance to, DANTSCHAKOFF's figure ('08, Fig. 1) which represents merely the germ-wall.

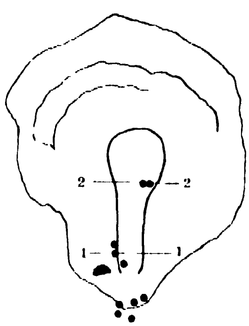

Fig. 3. Schematic representation of an embryo after a 10-hour incubation. ×30 Reconstructed. The primordial germ-cells observed are shown in black dots.

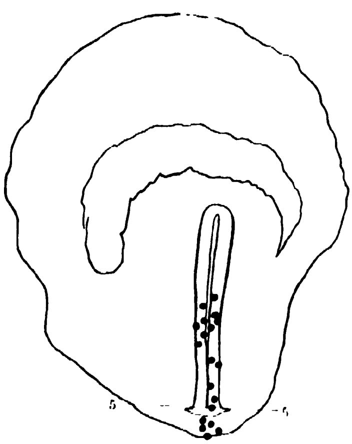

Fig. 4. Schematic representation of an embryo after a 14-hour incubation ×30. Reconstructed.

In the embryos, after from a 16-hour to an 18-hour incubation, in which the head process is forming, the primordial germ-cells are easily distinguishable from the neighboring somatic cells because of a diminution in their yolk contents and in the size of the somatic cells. Many of these germ-cells still in this stage are found in the cell-mass of the primitive streak, near the bottom of the primitive groove (Fig. 6) or sometimes even in the primitive groove itself attached to the bottom of it. This is probably due to a deepening of the primitive groove. It is also noteworthy that in this stage the germ cells are confined to the middle portion of the primitive streak (Fig. 7), owing to the addition of its posterior portion, while the shortening of its anterior portion has not yet begun.

Fig 5 Portion of the transverse section through 5–5 in Fig. 4. 14-hour incubation Picro-sublimate fixation and picro-carmine stain. ×700. Photographed. *ect* ectoderm, *ent* entoderm, *mes* mesodermal cells, *pg* primordial germ cell.

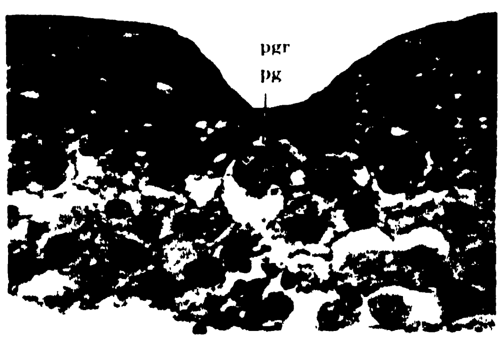

Fig. 6. Portion of the transverse section through 6–6 in Fig. 7. 18-hour incubation. Picro-sublimate fixation, and iron-hematoxylin and acid fuchsin stain. ×700. Photographed. *pg* primordial germ-cell, *pgr* primitive groove.

In the embryos after a 20-hour incubation, the mesodermal sheet is thickly formed just laterally to the cell-mass of the primitive streak, and some primordial germ-cells begin also to be found in it (Fig. 8). But even in the embryos after a 22-hour incubation, in which 3 or 4 pairs of somites have already been formed, most of them

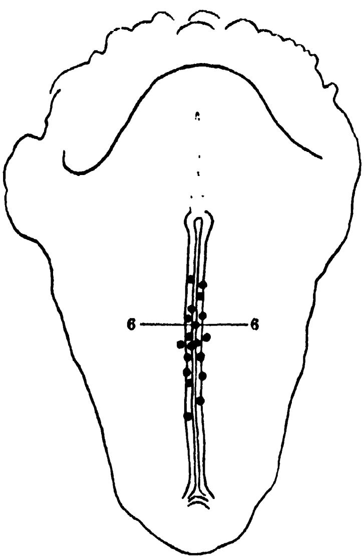

Fig. 7. Schematic representation of an embryo after an 18-hour incubation. ×20. Reconstructed.

still maintain their position in the regions of the primitive streak.

In 1908 certain large cells in the chick embryo which resemble the germ-cells were described by DANTSCHAKOFF. According to her, these cells appear at first in groups in the space between the ectoderm and germ-wall entoderm anterior to the formation of the embryo, during the stages from the primitive-streak to the 3-somite, before mesodermal tissue spreads into this region. They are large, containing a round nucleus and an abundance of yolk, and are specially characterized by their amoeboid properties. Because of this tendency she called them the entodermal " wander-cells ". These cells enter the mesoderm and forming blood-vessels, and, either by their own amoeboid powers or aided by the vascular circulation, penetrate into every part of the embryo and area vasculosa. Some of these entodermal wander-cells, leaving the blood-vessels by diapedisis, enter the embryonic tissues and then degenerate. Some undergo a similar fate in the blood-vessels themselves, and by the time the

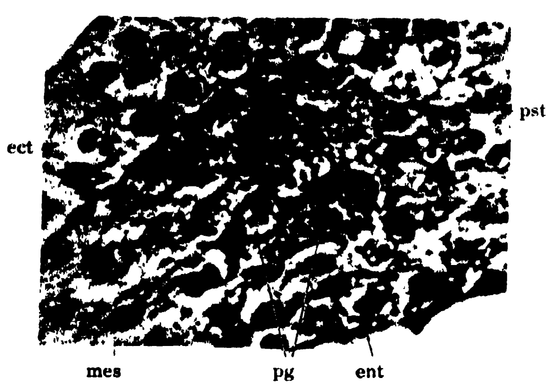

Fig. 8. Transverse section through the primitive streak of an embryo after a 20-hour incubation passing its posterior one-fourth, to show the primordial germ-cells in the mesodermal sheet just lateral to the embyronic axis. Picro-sublimate fixation, and iron-hematoxylin and acid fuchsin stain. ×700. Photographed. *ect* ectoderm, *ent* entoderm, *mes* mesodermal sheet, *pg* primordial germ-cells, *pst* primitive streak.

embryo has 22 somites nearly all disappear. Although the fate of those which do not degenerate is unknown, yet she believes that they have no share in tissue formation at all.

"These entodermal wander-cells of DANTSCHAKOFF", states SWIFT ('14), "are in reality the primordial germ-cells of the chick. There is a close agreement as far as origin is concerned, for I also find that the germ-cells originate from certain cells of the germ-wall entoderm near its junction with the area pellucida. I find also that they are produced during the primitive-streak stage, and in the embryo possessing at least 3 somites". Thus SWIFT describes only the agreement between the germ-cells and the entodermal wander-cells, without giving any information of the results of his direct observation except the lines, "That these particular germ-wall entodermal cells are producing the primordial germ-cells, is proven by the fact that in the primitive-streak stage, before the appearance of mesoderm anterior to the embryo, the germ-cells are grouped in the space between entoderm and ectoderm in this immediate neighborhood; the germ-cells cytologically are very similar to the cells of the germ-wall near its border; mitoses are also seen in these cells".

"The primordial germ-cells", states also GOLDSMITH ('28), "are first to be seen in the embryo during the primitive-streak stage. They are to be found at the outer edge of the proamnion, just at the margin of the zone of junction, anterior and antero-lateral to the head fold. They bud off from the inner portion of the entoderm and are found in the proamnionic area within the space between the entoderm and ectoderm before the mesoderm arises". As his primordial germ-cells, two cells are shown in his figures which represent the incubation stages of from 14 to 16 hours, one showing amoeboid characters and the other being in division. This sort of identification, however, appears to me to be very doubtful, for in my own materials all the so-called primordial germ-cells either in the primitive streak of the embryos after incubation from 14 to 16 hours or in the genital ridges of the embryos after incubation from 4 to 5 days never showed amoeboid characters, and it is so difficult to determine whether the cells in division are germ-cells or not, that these cases were omitted in my count of the number of germ-cells, a statement in relation to which will be found later. Again, in my observations, the entodermal wander-cells never contain attraction spheres and are also found even in the embryos of the 16-somite stage. Moreover, according to SWIFT and GOLDSMITH, the primordial germ-cells are produced in the germ-wall in front of the primitive streak during the primitive-streak stage and until

the embryo possesses at least 3 pairs of somites. But in my observations, also using embryos of similar stages as specimens, the primordial germ-cells are found confined to the region of the primitive streak. From these points I confess that I cannot believe that the entodermal wander cells of DANTSCHAKOFF can be identified with the primordial germ-cells. It may be stated here that EIGENMANN ('91) found the germ-cells in cross sections of a teleost fish through the anterior part of the embryonic axis before any proto-vertebrae were outlined, and according to DODDS ('10) the germ-cells are found in *Lophius* along the mediam line of the embryo proper in the earliest stages, in which the blastoderm has not yet covered half the yolk, and the formation of the embryo has only just begun.

At about the stage of the possession of 5 pairs of somites, after about a 23-hour incubation, the anterior end of the primitive streak begins to shorten as the embryo grows older. Consequently, the primordial germ-cells become distributed in the anterior half and adjacent region of the primitive streak (Fig. 10), and are found even in the basal cell-mass of

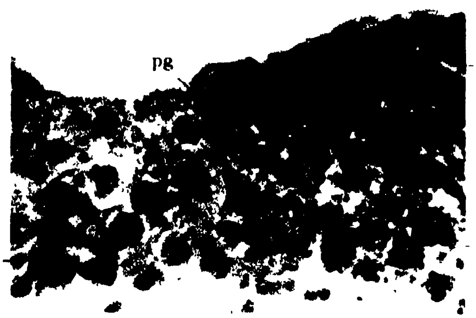

Fig. 9. Transverse section through the basal cell-mass of the head process of an embryo after a 24-hour incubation, passing 9–9 in Fig. 10. Fixation and staining as before. × 700 Photographed. *ect* ectoderm now transforming into the posterior region of the medullary plate, *hp* cell mass of the head process, *mes* right mesodermal sheet, *pg* primordial germ-cell.

the head process, mingled with the mesodermal and ectodermal cells (Fig. 9); the latter cells are now forming the posterior portion of the medullary plate.

In the embryos which have 8 to 10 pairs of somites, after from a 26 to 30-hour incubation, nearly all the primordial germ-cells are forced aside from the anterior portion of the primitive streak into the adjacent meso-

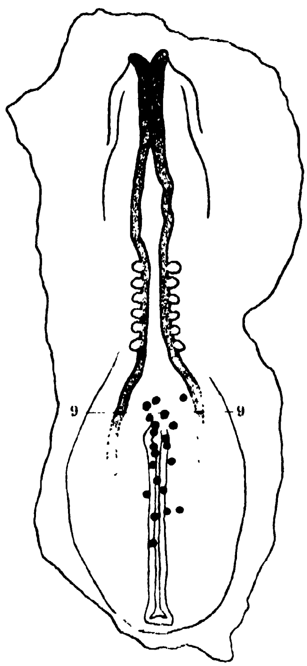

Fig. 10 Schematic representation of an embryo after a 24-hour incubation (6-somite stage) ×20 Reconstructed.

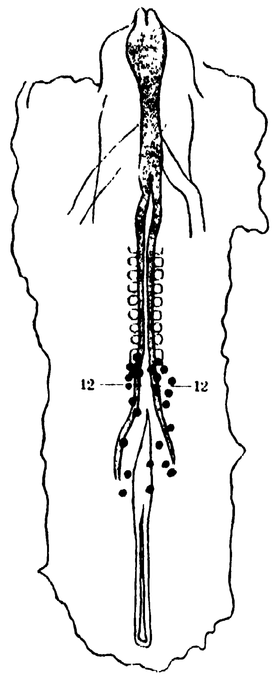

Fig. 11. Schematic representation of an embryo after a 26-hour incubation (8-somite stage). ×20. Reconstructed.

dermal sheet owing to the displacement of the primitive tissues. The foremost ones then become contained in the mesodermal sheet, which is just caudal to the last somite (Fig. 11) and now has a loose appearance in sections, being situated a little remote from the proximal portion of the head process (Fig. 12).

As a brief summary of this chapter, it may be stated that the primordial germ-cells, which are found in the middle and posterior region of the primitive streak as well as in the primitive plate in the stage of a 10-hour incubation, are gradually transported with the lapse of time to the middle region, then to the anterior region, and finally to the mesodermal sheet owing to the displacement of the primitive tissues, but not to their active movement; and that in the 8 or 10-somite stage nearly all the primordial germ-cells are distributed in the mesodermal sheets just lateral to the embryonic axis and just caudal to the last pair of somites.

From the facts above stated it may be presumed that the primordial

Fig. 12. Transverse section through the proximal portion of the head process of an embryo after a 26-hour incubation, passing 12–12 in Fig. 11, to show a primordial germ-cell in the left mesodermal sheet. Its position is a little remote from the embryonic axis. Picro-sublimate fixation, and iron-hematoxylin and acid fuchsin stain. ×700. Photographed. *ect* ectoderm, *ent* entoderm, *mes* mesodermal sheet, *pg* primordial germ-cell

germ-cells are localized in the posterior region of the germ-ring in the stage prior to a 10-hour incubation, and are transported to the primitive streak probably as a result of the confluence movement of tissue materials.

PRIMORDIAL GERM-CELLS IN THE STAGES FROM A 33-HOUR TO A 4-DAY INCUBATION.

In the stage of a 33-hour incubation, the number of somites increases to 12 pairs, and the vitelline arteries are forming laterally to the dorsal aortae just in front of the anterior termination of the region where the primordial germ-cells are distributed, and even in this "germ-cell region" the mesodermal sheets begin to divide into the somatic and splanchnic mesodermal layers. In this stage the germ-cells begin to be found in some numbers in the mesodermal sheets being carried outwards from the positions which they formerly occupied, owing mainly to the lateral extension of the median portion of the mesoderm (Fig. 13). In this stage, however, some of the more posterior germ-cells may also be still found even in the basal and adjacent portion of the head process.

In the embryos in the 16-somite stage after a 38-hour incubation, in which the separation of the mesodermal layers is more or less spread

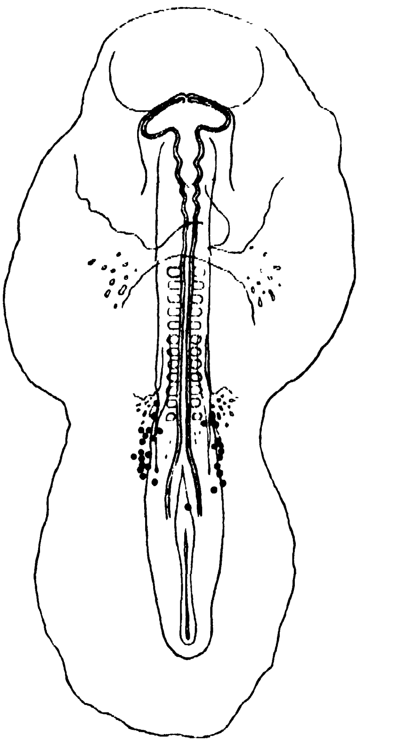

Fig. 13 Schematic dorsal view of an embryo after a 33-hour incubation (12-somite stage) to show the distribution of primordial germ-cells ×15 Reconstructed.

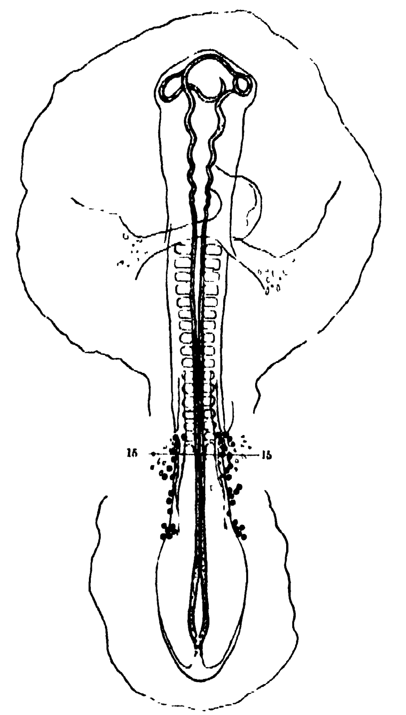

Fig. 14. Schematic dorsal view of an embryo after a 38-hour incubation (16-somite stage) to show the distribution of primordial germ-cells. ×15. Reconstructed

towards the embryonic axis, and the somite formation begins to proceed even in the germ-cell region posterior to the forming vitelline arteries, the distribution of primordial germ-cells appear to be nearly the same as that in the 12-somite stage (Fig. 14), still being found inside the coelomic angle, where the somatic and splanchnic mesoderms are continuous with the intermediate cell-mass or with the undifferentiated median portion of the mesodermal sheet (Fig. 15).

In the embryos in the 20-somite stage after a 42-hour incubation, the position of the primordial germ-cells becomes further remote from the embryonic axis, and on either side of the body, as the coelomic angle proceeds or the separation of the mesodermal layers spreads towards the axis, most of the primordial germ-cells are forced to occupy a position

Fig. 15. Portion of the transverse section passing the anterior portion of the germ cell region just through 15–15 in Fig. 14. 38-hour incubation. Picro-sublimate fixation, and iron-hematoxylin and acid fuchsin stain. ×700. Photographed. This photograph shows in fact the tissues on the right side of the embryo, but not on the left. *ca* coelomic angle at present, *ect* ectoderm, *ent* entoderm, *pg* primordial germ-cells in the right mesodermal sheet, *som* somatic mesoderm, *spl* splanchnic mesoderm.

either in the somatic mesoderm or in the splanchnic mesoderm adjacent to the respective coelomic angle, while some still remain in the mesodermal sheet (Figs. 16 and 17). Consequently in this stage, in the anterior portion of the germ-cell region most of the primordial germ cells are found in

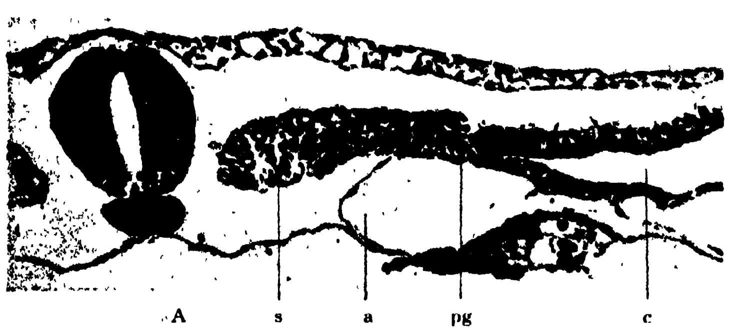

Fig 16 A Portion of the transverse section through 16 16 in Fig 18. 42-hour incubation. Fixation and staining as before. ×200. Photographed. Abbr. see B.

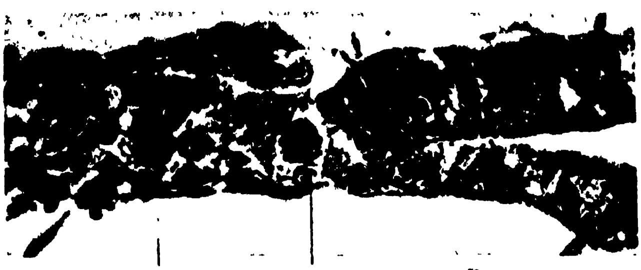

Fig. 16 B. The same as A. ×700. Photographed. *a* right dorsal aorta, *c* coelom, *pg* primordial germ-cell still in the mesodermal sheet, *s* somite.

the neighborhood of the coelomic angle and in the mesodermal layers, though they are more numerous in the splanchnic mesoderm than in the somatic mesoderm. In the posterior portion most of them are still contained

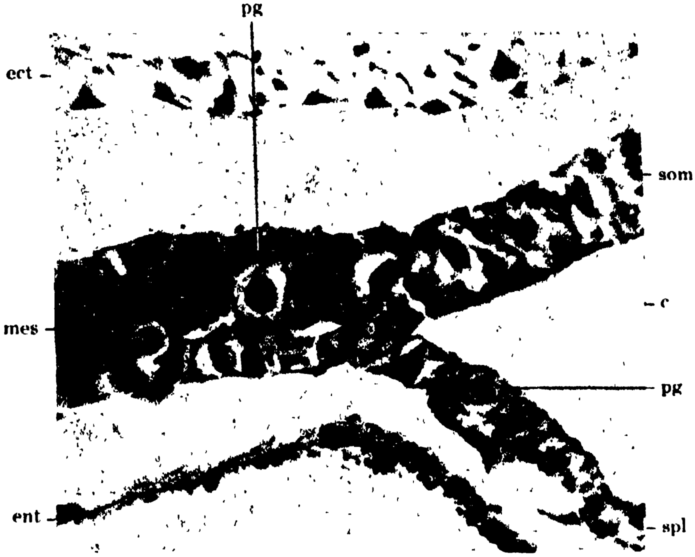

Fig. 17. Portion of the transverse section through 17–17 in Fig. 18 42-hour incubation. Fixation and staining as before. ×700. Photographed. The separation of the mesodermal layers is still spreading toward the embryonic axis. *c* coelom, *ect* ectoderm, *ent* entoderm, *mes* right mesodermal sheet, *pg* primordial germ-cells, *som* somatic mesoderm, *spl* splanchnic mesoderm.

in the mesodermal sheet in which the separation of the layers has not yet been completed (Fig. 18).

SWIFT ('14) observed in an embryo with 19 pairs of somites that the

primordial germ-cells are found in the blood-vessels, in the large as well as in the small channels both of the area vasculosa and embryo proper, and are present even in the heart and aortae, possessing amoeboid processes in nearly all cases. "The stages of 16, 12 and 10 somites respectively," states SWIFT, "may be passed rapidly since they present nothing essentially different from the 19 somites embryo as far as either the distribution or form of the germ cells is concerned. Thus, the latter are found in the area vasculosa and in the developing vascular structures of the embryo proper. In the embryo possessing 12 somites, one germ-cell in particular is found in the heart."

Von BERENBERG-GOSSLER ('14) confirms SWIFT's statement by his own

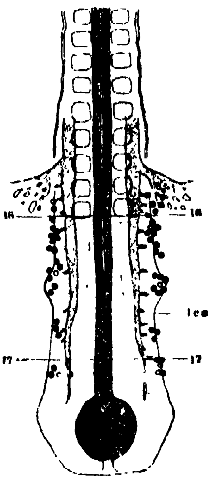

Fig. 18 Schematic dorsal view of the posterior embryonic body after a 24-hour incubation (20-somite stage). ×25 Reconstructed. *lca* innermost limit of the coelomic angle formed at present White dots indicate germ-cells which show a tendency to be contained or are contained already in the somatic mesoderm, while black dots indicate those which show a tendency to be contained or are contained already in the splanchnic mesoderm

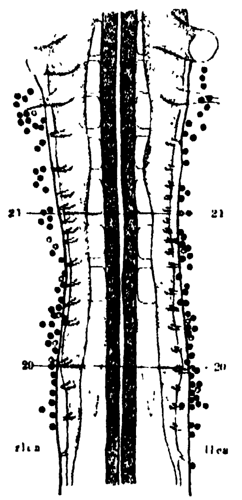

Fig. 19 Schematic ventral view of the germ-cell region in the stage of a 50-hour incubation. ×50 Reconstructed. *lca* innermost limit of the coelomic angle traced in this stage

observation and states that the primordial germ cells appear within the vascular channels in the chick embryo prior to the 25-somite stage. GOLDSMITH ('28) also states that no primordial germ-cells can be found in the embryo proper during the period up to about a 33-hour incubation, in

about the 12-somite stage, although they are relatively common in the extra-embryonic region.

Thus according to SWIFT, von BERENBERG-GOSSLER and GOLDSMITH, even in the 20-somite stage mentioned above, the primordial germ-cells ought to be found also only in the blood-vessels or in the space formed in the extra-embryonic region, but in fact, in no embryo investigated by the present writer was such a case encountered.

In the embryos after a 50-hour incubation, the primordial germ-cells are also found in the splanchnic mesoderm in greater numbers than in the somatic mesoderm (Figs. 19 and 20) though a small number remain inside the coelomic angle which is now fully formed; in some cases they may be found in the splanchnic mesoderm at some distance from the coelomic angle. In fact, HOFFMANN ('92), NUSSBAUM ('01), SWIFT ('14), GOLDSMITH ('28) and others, nearly all who support the theory of early segregation in birds, in reference to the stages later than those of a 45-

Fig. 20. Portion of the transverse section through 20–20 in Fig. 19. 50-hour incubation. Picro-sublimate fixation, and iron-hematoxylin and acid fuchsin stain ×700. Phot graphed. *a* right dorsal aorta, *ca* coelomic angle, *itm* intermediate cell-mass, *pg* primordial germ-cell, *som* somatic mesoderm, *spl* splanchnic mesoderm.

hour incubation prior to the formation of the genital ridge, state that the primordial germ-cells are found gathering in the splanchnic mesoderm; and in addition to this SWIFT and GOLDSMITH assert that some still remain in the blood stream. Indeed, at this stage, the present writer also met with two cases of the presence of a large cell in the blood-vessel, one in

a small channel and the other in the dorsal aorta (Fig. 21). These large cells are exactly identical with the primordial germ-cells, and these are the only cases met with in the course of his observations. It is quite possible therefore that some primordial germ-cells may issue by chance from the splanchnic mesoderm together with the endothelial cells and enter the blood stream.

Fig. 21. Portion of the transverse section through 21 21 in Fig 19. 50-hour incubation. Fixation and staining as before. ×700. Photographed Abbreviations as before.

In connection with the stages of from a 38-hour to a 50-hour incubation, it may be stated here that the germ-cell region gradually extends antero-posteriorly with the increase of length of the embryo. In all embryos, however, the anterior extent is always exactly coincident with the posterior border of the pair of vitelline arteries, while the posterior limit appears to be almost coincident with the caudal end of the developing dorsal aortae (Figs. 14, 18 and 19).

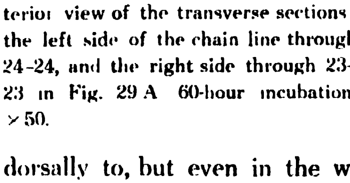

Fig. 22. Combined schematic posterior view of the transverse sections: the left side of the chain line through 24-24, and the right side through 23-23 in Fig. 29 A 60-hour incubation. ×50.

After a 60-hour or a $2\frac{1}{2}$-day incubation, the blastocoelic space in the embryos is almost filled with mesenchymal cells not only in the body wall situated dorsally to, but even in the walls of the mid-gut situated ventrally to, the coelomic angles, which stand apart from each other forming at that time

a wide radix mesenterii between them; and the dorso-lateral walls of the mid-gut, which may now be called the splanchnic plates, are spread nearly

Fig. 23. Portion of the transverse section through 23–23 in Fig. 29 A, just showing the rectangle 23 in Fig 22. 60-hour incubation Picro-sublimate fixation, and iron-hematoxylin and acid fuchsin stain. ×700 Photographed. *c* coelom, *ent* entoderm, *g* mid-gut, *ra* right dorsal aorta, *pg* primordial germ-cell, *som* somatic mesoderm, *spl* splanchnic mesoderm

horizontally (Figs. 22 and 29 A). The posterior end of the dorsal aorta goes far beyond the posterior extent of the germ-cell region, and in this

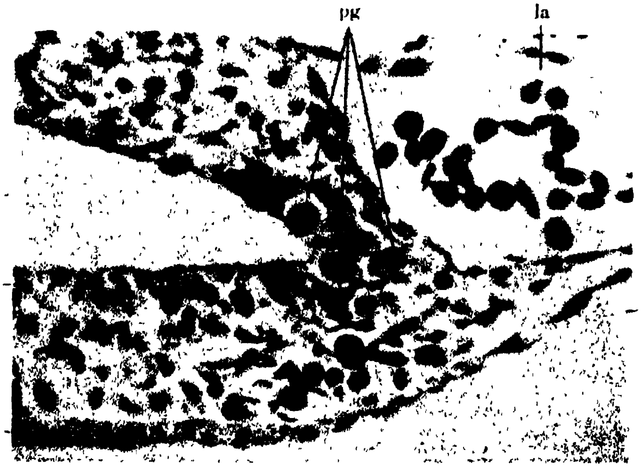

Fig. 24. Portion of the transverse section through 24–24 in Fig. 29 A, just showing the rectangle 24 in Fig. 22. 60-hour incubation. Fixation and staining as before. ×700. Photographed. *la* left dorsal aorta. Other abbreviations as before.

region 8 pairs of fully distinguished somites may be counted. In this stage too, most of the primordial germ-cells still maintain their position in the mesodermal layers, both somatic and splanchnic, and in sites of the coelomic angles (Figs. 23 and 24). SWIFT ('14) also observed and describes similar conditions. "In embryos with 30 to 33 somites the primordial germ-cells are in the radix mesenterii and coelomic epithelium on both sides of the coelomic angle. They remain in this position until the formation of the gonad begins, when they gradually pass into that organ." Moreover it is worthy of note that the surface of the somatic as well as the splanchnic mesoderm towards the blastocoelic space becomes obscure because of the liberation of the mesenchymal cells which may also accompany the germ-cells.

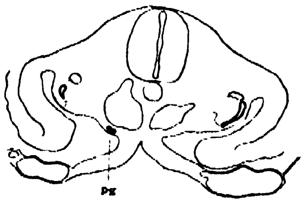

Fig. 25. Schematic transverse section through 25–5 in Fig. 29 B. 72-hour incubation. ×50. *pg* primordial germ-cells.

In fact, in the embryos after a 3-day incubation, the primordial germ-cells are scattered even in the mesenchymal spaces, sometimes adjacent to the entoderm, but they are found most frequently in the space adjacent to the coelomic epithelium, which is now beginning to be distinguished clearly from the mesenchyme (Fig. 27), and contains also many primordial germ-cells (Fig. 26). In this stage, the radix mesenterii becomes so much narrower than that of the $2\frac{1}{2}$-day

Fig. 26. Portion of the transverse section through 26–26 in Fig. 29 B. 72-hour incubation. Picro-sublimate fixation, and iron hematoxylin and acid fuchsin stain. ×700. Photographed. *c* coelom, *la* left dorsal aorta, *pg* primordial germ-cell in forming genital ridge.

stage, that the primordial germ-cells on both sides of it are drawn nearer in comparison with their position in the latter stage (Figs. 29 A and B) and the dip of the splanchnic plates becomes acute or nearly vertical (Fig. 25), especially in the posterior half of the germ-cell region where there appears a transition to the hind-gut (Fig. 27). In the germ-cell region also 8 pairs of well-defined somites are present. The vast majority of the primordial germ-cells are found in this stage on the site of the outer angles of the radix mesenterii, being equally distributed on the dorsal and ventral sides of it, even though some may be found in the splanchnic plate considerably apart from that region. As to the presence of so many primordial germ-cells equally on both sides of either angle of the radix mesenterii, in stead of being present in the slanchnic mesoderm in greater number than in the somatic mesoderm, as stated in connection with the 50-hour stage, I am of opinion that the original coelomic angle undergoes a slight displacement dorsalwards towards the mesonephros or its duct

Fig. 27. Portion of the transverse section through 27–27 in Fig. 29 B 72-hour incubation. Fixation and staining as before. ×700. Photographed. *g* mid-gut, *ra* right dorsal aorta, *som* somatic peritoneum. Other abbreviations as before.

along with the tissues representing a narrow proximal portion of the splanchnic mesoderm of the early stages and disappears. Consequently, the present angle of the radix mesenterii is not in any case the original coelomic angle but is a new formation which is occasioned in the original tissue of the splanchnic mesoderm where the richest of primordial germ-cells are

present. Moreover, I may mention here that in my observations of an embryo in this stage I succeeded in finding a few primordial germ-cells in the mesenchyme between the dorsal aorta and the Wolffian duct which is remote from the future genital ridge (Fig. 28). These cases are in my opinion due to the occasional presence of the primordial germ-cells in the original intermediate cell-masses.

Fig. 28. Portion of the transverse section through 28-28 in Fig. 29 B, to show a primordial germ-cell in the mesenchymal space between the dorsal aorta and the Wolffian duct. 72-hour incubation. Fixation and staining as before. ×700. Photographed. *lcv* left posterior cardinal vein, *md* left Wolffian duct Other abbreviations as before.

In the embryos in the $3\frac{1}{2}$-day stage, 9 pairs of definitely-formed somites may be counted in the germ-cell region; the mesentery is forming with the progress of the downward movement of the mid-gut entoderm; the subcardial veins are distinctly formed; and the union of the left and right dorsal aortae is completed in the anterior portion of the germ-cell region, even though sometimes longitudinal septa are found between them in its posterior portion. Moreover, in this stage the so-called genital ridge is forming on either side of the radix mesenterii (Figs 30 and 31). In reality, from my own observation, the tissue of the genital ridge at this stage covers not only the bulge just lateral to the outer angle of the radix

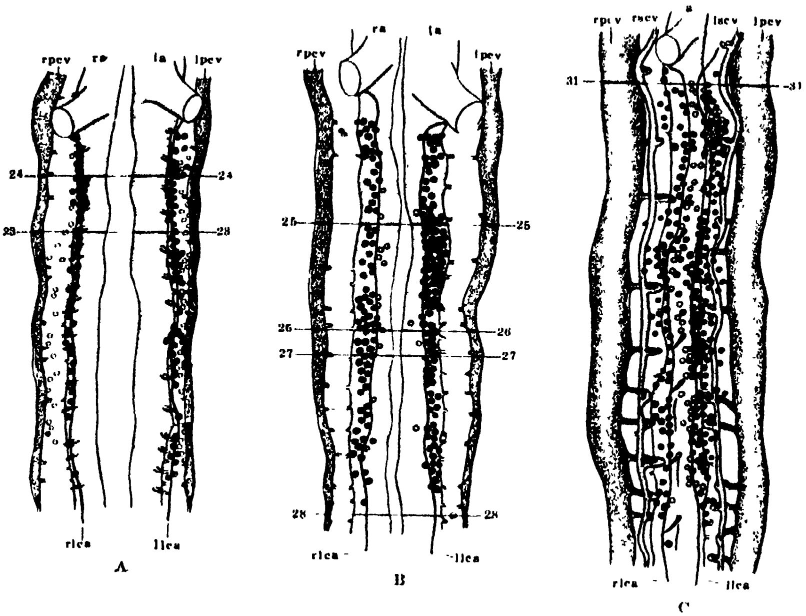

Fig. 29. Schematic ventral views of the germ-cell region at different stages, to show the interrelation between positions of arteries, veins, primordial germ-cells and radix mesenterii. ×50. Reconstructed. Since the germ cell region is curved in these stages being effected by the downward curvature of the caudal portion of the embryo, the projection of the antero-posterior extent of the germ-cell region is shown in this figure somewhat shorter than that in Fig. 19. in which it is not yet curved.

A. 60-hour stage Distances of primordial germ-cells from the coelomic angle are distinguished for convenience by different dots, black ones showing those which are placed relatively near the coelomic angle, and white ones those which are placed relatively far from the coelomic angle. White dots with nucleus denote the primordial germ-cells which are found in the mesenchymal space adjacent to the dorsal aorta and mesonephros or its duct. *l* - *ra* left and right dorsal aortae, *l* — *rlca* lines which denote the left and right coelomic angles, *llca*-*rlca* radix mesenterii. *l* — *rpcv* left and right posterior cardinal veins.

B. 72-hour stage. Relations between dots and distances are the same as in A, but in this case the coelomic angles have already disappeared, so that the coelomic angle means the outer angle of the radix mesenterii. The number of black dots is increased owing to the displacement and disappearance of the original coelomic angle, because this accompanies also the displacement of the original splanchnic tissue which contains many primordial germ-cells. Abbreviations as before.

C. 84-hour stage. Black dots denote the primordial germ-cells found in the forming genital ridge, white dots those in the forming mesentery, and white dots with nucleus those in the mesenchymal space adjacent to the dorsal aorta and the mesonephros or its duct. *a* united dorsal aorta, *l* — *rscv* left and right subcardial veins. Other abbreviations as before.

mesenterii, but even the portion of the mesentery just ventrally situated to it, this statement being supported by the similarity of tissue appearance and by the presence of abundant primordial germ-cells (Fig. 32). In my opinion, as already stated, the tissue of the genital ridge is originally that of the splanchnic mesoderm which is displaced passing over the original coelomic angle and becoming adjoined to the innermost somatic mesodermal tissue. This tissue also contains primordial germ-cells, though the number is less than that in the splanchnic mesoderm. The original coelomic angle is involved in the surface of the bulge of the genital ridge as it is forming.

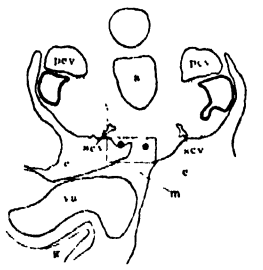

Fig. 30. Portion of a transverse section through the anterior portion of the germ-cell region. 84-hour incubation. ×50. *a* dorsal aorta, *c* coelom, *g* mid-gut, *m* mesentery, *pcv* posterior cardinal vein, *scv* subcardinal vein

In the embryos in the 4-day stage, 10 pairs of clearly defined somites are counted in the germ-cell region; the union of the dorsal aortae has been completed and the genital vein has been distinctly formed inside the respective genital ridge. The tissue of the genital ridge remains still covering the dorsal or proximal

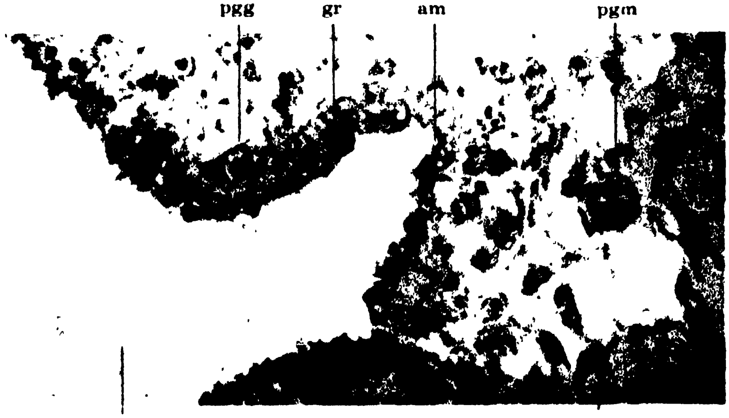

Fig. 31. Portion of the transverse section shown in the rectangle of Fig 30. 84-hour incubation. Picro-sublimate fixation, and iron-hematoxylin and acid fuchsin stain. ×700. photographed. *am* left outer angle of the radix mesenterii formed amidst the surface of the forming genital ridge, *c* coelom, *gr* forming genital ridge, *m* forming mesentery, *pgg* primordial germ cell already in the site of genital ridge, *pgm* same contained in the mesenchyme of mesentery.

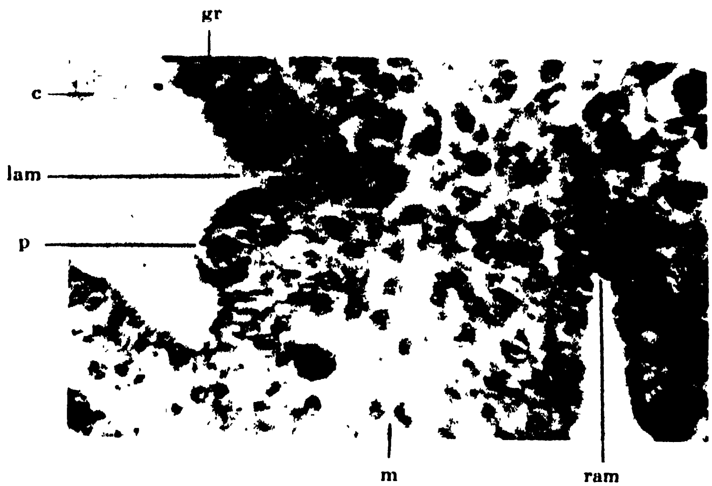

Fig. 32 Portion of a transverse section through the posterior portion of the germ-cell region. 84-hour incubation. Fixation and staining as before. ×700. Photographed. *c* coelom, *gr* forming genital ridge, *l* *ram* left and right outer angles of the radix mesenterii. *m* forming mesentery, *pg* primordial germ-cell contained in the tissue of genital ridge covering the forming mesentery.

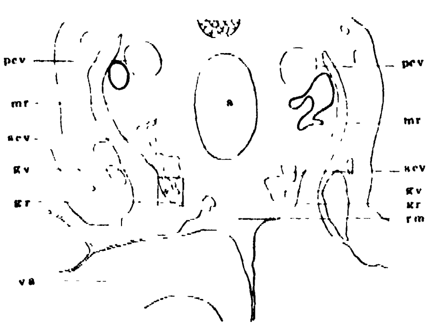

Fig. 33. Schematic representation of a portion of the transverse section through the foremost portion of the germ-cell region adjacent to the vitelline artery. 96-hour incubation ×50 *a* dorsal aorta, *gr* genital ridge, *gv* genital vein, *mr* mesonephric region *pcv* posterior cardinal vein, *rm* radix mesenterii, *scv* subcardinal vein *va* vitelline artery.

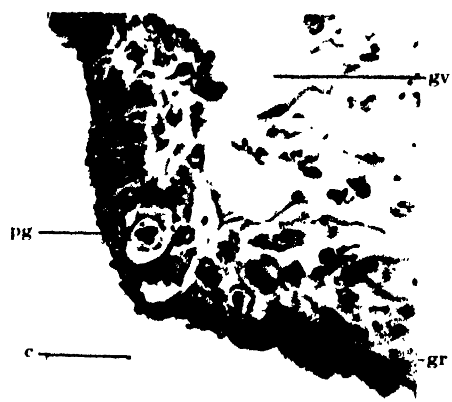

Fig 34 Portion of the transverse section just showing the rectangle in Fig. 33. 96-hour incubation. Picro-sublimate fixation, and iron-hematoxylin and acid fuchsin stain. ×700. Photographed. *c* coelom, *gr* genital ridge, *gv* genital vein, *pg* primordial germ-cell in the genital ridge.

surface of the mesentery in the major posterior portion of the germ-cell region (Fig. 35), but in the foremost portion adjacent to the vitelline artery the whole tissue has already passed over the outer angle of the radix mesenterii and is laid laterally along it (Fig. 33).

Fig. 35. Portion of a transverse section through the posterior portion of the germ-cell region. 96-hour incubation. Fixation and staining as before. ×350. Photographed. *a* dorsal aorta, *c* coelom, *l — rca* left and right outer angles of the radix mesenterii, *m* mesentery, *pgd* primordial germ-cell in division in doubt, *pgdd* primordial germ-cells in the mesenchyme dorsal to the radix mesenterii, *pgm* the same in the mesentery.

In both the $3\frac{1}{2}$-day and 4-day stages, the primordial germ-cells are found in great abundance both in the epithelium and in the adjacent mesenchymal spaces (Figs. 31, 32, 34 and 35), but sometimes they are also found in the mesenchyme situated dorsally to the radix mesenterii (Fig. 35). The latter cases are, in my opinion, due to the dorsalwards displacement of the mesenchymal tissue, which was placed formerly in the dorsal portion of the mesentery, accompanied by the displacement of the

[illegible] of the forming genital ridge. In the mesentery also are found a few primordial germ-cells, the sites of which are at a considerable distance from the radix mesenterii. These cells are probably those which underwent further movement when the mesodermal sheet extended laterally, as pointed out in connection with the 50-hour stage. Thus, up to these stages, I think, the primordial germ-cells never change their position by their own active migration, but are moved to their position passively by the displacement of the tissues containing them.

Again, in a brief summary of this chapter, it may be mentioned here that the primordial germ-cells, which were distributed in the mesodermal sheet during the earlier stages of the embryo, move somewhat further outward from the embryonic axis while the median portion of the mesodermal sheet continues to extend laterally, and when the mesodermal sheet divide, a larger number of them are eventually contained in the splanchnic mesoderm and a smaller number in the somatic mesoderm. In about the 3½-day stage, the tissues, both epithelium and mesenchyme, which were originally those of the splanchnic mesoderm, begin to be displaced dorsalwards together with those containing the primordial germ-cells and become adjoined to those of the somatic mesoderm. Thus the development of the genital ridge begins. As to the destination of those primordial germ-cells which are found in places at a considerable distance from the genital ridge, I am unable to say anything at present.

Finally, as may be seen from my statements given above, I could find no evidence in the chick embryo in support of either the gonotome theory which was held by RÜCKERT ('88) and van WIJHE ('89) in reference to selachia and was supported by HALL ('04), SPEHL and POLUS ('12), SCHAPITZ ('12), CHAMPY ('13), and BECCARI ('22) all in reference to amphibia, or of the entoderm theory as observed by BEARD ('00, '02) in selachia and *Petromyzon*, by ALLEN ('09) in *Amia*, and by KOHNO ('25) in *Homo*.

NUMBER OF THE PRIMORDIAL GERM-CELLS

Some investigators have made statistical studies in regard to the number of the so-called primordial germ-cells during the early stages of vertebrate development. EIGENMANN ('96) in *Cymatogaster* and BEARD ('02) in *Raja* and other animals agree absolutely on the point that for a long period in the early development of the embryo, the sex-cells do not divide, the number remaining constant with the exception of the degeneration of a very few. ALLEN ('07) citing these two investigators states that no figures

of mitoses are observed in the sex-cells of the embryo of *Chrysemys* longer than 2.8 mm. in total length, and the first clear indications of their division are found in the embryo which has a body length of 10 mm., though some cases where there has been no division may be found even in stages later than this. Later ALLEN ('09) states again in the work on *Amia* and *Lepidosteus* that "the absence of indications of cell-divisions, during the migration process, is characteristic of the sex-cells of all vertebrates studied." DODDS ('10) states in the study of *Lophius* that "the 'period of rest' of the germ-cells begins before the separation of the mesoderm and continues until after hatching." Recently WOLF ('31) has also pointed out that there is a constancy in the number of germ-cells in the early stages of *Platypoecilus*.

In order to know whether the primordial germ-cells multiply or not in the early stages of the chick embryo, I made a careful count of their number in 25 embryos at 15 different stages, while I was determing their distribution. Some plottings of these have already been shown in Figs. 3, 4, 7, 10, 11, 13, 14, 18, 19, and 29, each figure being prepared from each single individual. It is quite evident, however, that the number and arrangement of the germ-cells are never alike in different individuals even of the same age, as may be seen in Table 2.

In Table 2, the number of germ-cells given is that of two different individuals in the earlier stages and of one only in the later stages. I also met with many mitotic figures of probable germ-cells in all and especially in later stages, but these cases were all omitted from the count, because of difficulties in distinguishing them from somatic cells in division.

TABLE 2.

Hours of incubation	10	12	14	16	18	24	26	33	38	42	50	60	72	84	96
No. of Fig.	3		4		7	10	11	13	14	18	19	29 A	29 B	29 C	
No. of the primordial germ-cells observed.	10	13	19	17	19	21	26	31	38	54	115	166	214	299	421
	9	8	12	16	22	18	25	34	40	46					

The apparent increase in the number of germ-cells, which Table 2 reveals, may be due to some difficulty of observation especially in the early primitive-streak stage, as DODDS ('10) states that "the apparent

increase, during certain early stages, is due to changes in the germ-cells which make them recognizable." Nevertheless, in conclusion from the general tendency which my data suggest, I may give it as my view at present, that a gradual multiplication of germ-cells may be continued in the chick embryo even in the stages of from a 10-hour to a 4-day incubation, and even if this is not the case in other animals.

SWIFT ('14) and GOLDSMITH ('28) have also observed the mitotic figures of primordial germ-cells in the early stages of the chick embryo, but in the vascular channels, so that these cases are of course outside the scope of my argument.

SUMMARY.

In this study of the chick embryo during the stages of from a 10-hour to a 4-day incubation, the original position and path of movement of the so-called primordial germ-cells have been discovered. The results arrived at are quite different from those put forward by SWIFT and GOLDSMITH.

1. The posterior margins of the germ-ring are suggested as the original situation of the primordial germ-cells in the stages prior to a 10-hour incubation.

2. The primordial germ-cells are found in the posterior portion of the primitive streak including the primitive plate, during the stages of from a 10-hour to a 11-hour incubation.

3. They are transported anteriorly together with the cell-mass of the primitive streak with the increase in lentgh of its posterior portion, during the stages of from a 16-hour to a 22-hour incubation.

4. The primordial germ-cells reach their position in the anterior portion of the primitive streak with the progress of its anterior shortening, during the stages of from a 23-hour to a 26-hour incubation.

5. They are given off into the median portion of the lateral mesodermal sheet while it is forming on either side of the head process, during the stages of from a 26-hour to a 30-hour incubation.

6. When the lateral mesodermal sheet separates into two mesodermal layers, a larger number of the primordial germ-cells are arranged in the splanchnic mesoderm, and a smaller number in the somatic mesoderm, during the stages of from a 33-hour to a 50-hour incubation.

7. While mesenchymal tissue is forming, a larger number of primordial germ-cells are again liberated into the spaces of this tissue which are adjacent to the coelomic epithelium. The tissue then contains merely the

remaining small number of germ-cells and is beginning to be distinguished from the mesenchymal tissue, during the stages of from a 60-hour to a 72-hour incubation.

8. Both the mesenchyme and coelomic epithelium, which were originally part of the splanchnic mesoderm, contain a vast majority of the primordial germ-cells. These tissues begin to displace dorsalwards, passing over the outer angle of the radix mesenterii, and adjoin those of the oringnal somatic mesoderm which also contains many germ-cells. Thus, the genital ridge begins to form, during the stages of from an 84-hour to a 96-hour incubation.

9. It is suggested that the multiplication of primordial germ-cells begins even during the stages of from a 10-hour to a 96-hour incubation, but that no active migration of primordial germ-cells occurs until the stage of a 96-hour incubation has been reached.

10. As to the destination of those primordial germ-cells which are found in positions at a considerable distance from the genital ridge, I am unable to come to any conclusion at present.

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Pollen-Analytical Studies of Peat Formed on Volcanic Ash.[1)]

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(With 2 text-figures)

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Recently a report was published by Prof. YOSHII[2)] with his co-worker on the remarkable grass-moor on Mt. Hakkôda, which has developed on volcanic ash. He suggested and also provided the writer with the material on the analytical research of peat there formed. The present paper is a general account of pollen-analytical investigation of the peat.

In our country no research has ever been made along this line, and descriptions of the pollen of our plants are also lacking. Therefore, I have made investigations on the pollen of our main forest trees for the determination of the fossil pollen found in our country. On the basis of the data thus obtained, I am making pollen-analyses of peats collected from northern Japan. The present study concerns only a part of the peats formed on Mt. Hakkôda.

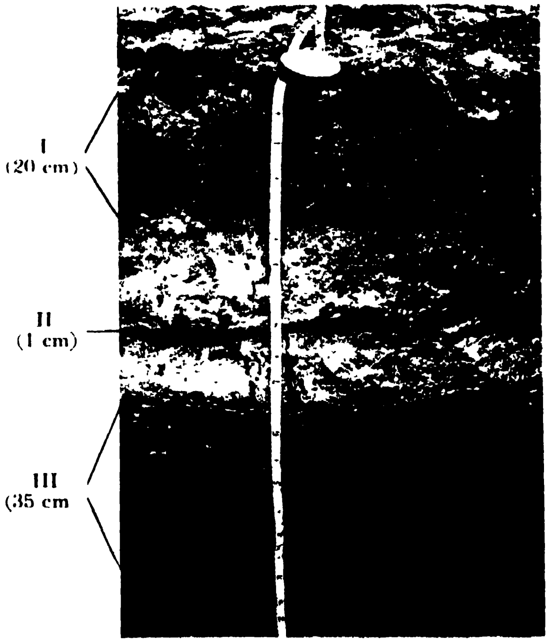
I will refer briefly to the nature of the moor in question according to the work mentioned above: The grass-moor, predominated by *Molinia japonica*, covers terraces on the western slope of the mountain at a height of about 1100 metres above sea-level and is characterized by a number of peculiar small ponds scattered on it. It is remarkable that this moor develops on deposit of volcanic ash, under which lie several layers of peat, intercalated by layers of volcanic ash formed by repeated eruptions. The photograph shows a profil of the moor-ground. The first peat layer is about 20 cm deep, the second peat layer is only 1 cm in thickness, while the third one is as thick as 35 cm.

Samples for pollen-analyses were taken from different levels in each layer of peat. Results obtained are shown in the following table, in which the frequency of the pollen found at different levels is expressed as percentages of the total pollen. Since practical difficulties are often encountered

[1)] Contributions from the Mt. Hakkôda Botanical Laboratory. No. 13.

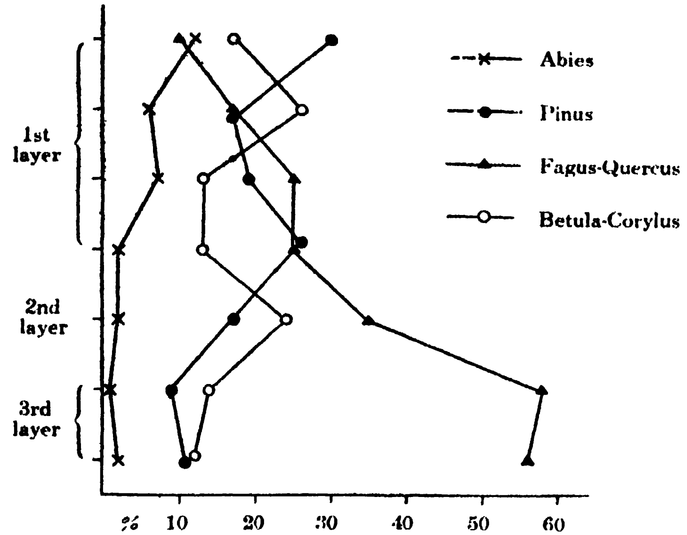
[2)] YOSHII, Y., und HAYASI, N. 1931. Botanische Studien subalpiner Moore auf vulkanischer Asche. Sci. Rep. Tôhoku Imp. Univ. 4th Ser. (Biol.) Vol. 6. p. 307.

on distinction between the pollen of *Fagus* and of *Quercus*, as well as between those of *Betula* and of *Corylus*, they were here counted together, respectively. However, so far as my examination could prove, it appeared, that *Fagus*-pollen predominated over *Quercus*-pollen, and that *Betula*-pollen surpassed the *Corylus*-pollen.



A profil of the moor-ground I, II, III are peat layers.

		Abies	*Pinus*	*Fagus-Quercus*	*Betula-Corylus*	*Alnus*	*Carpinus*	*Ulmus*	*Acer*	*Salix*
1st layer	Upper part	12	30	10	17	14	0	6	9	2
	Middle part (upper)	6	17	17	26	5	1	6	20	2
	" " (lower)	7	19	25	13	12	6	9	6	3
	Lower part	2	26	25	13	3	4	10	15	2
2nd layer		2	17	35	24	4	1	3	12	2
3rd layer	Upper part	1	9	58	14	5	3	4	4	2
	Middle part	2	11	56	12	5	0	12	1	1



The main points from the table are represented by the diagram.

It is remarkable that *Abies* is found in a larger number in the upper layer, being very scarce in the lower ones. *Fagus* and *Quercus*, which are considerably abundant in the third layer, decrease remarkably in the upper layers. *Pinus* increases in the upper layer, while *Betula* (including *Corylus*) remains rather constant. Although both trees show higher percentages, it must be noted that they, particularly *Pinus*, produce a great amount of pollen in nature. *Alnus*, *Ulmus* and *Acer* show no feature worth noting, and *Carpinus* and *Salix* are of less importance. The most remarkable fact is that *Abies* has increased in recent time, while *Fagus* and *Quercus* have been decreasing remarkably.

In connection with these facts represented in the results of pollen-analyses, reference must be made to the present aspect of the vegetation in the environment of the moor. As was described by Dr. HORIKAWA[1], a large area of the upper part of Mt. Hakkôda is covered by forests of *Abies Mariesii*. Conifers on this mountain are represented almost exclusively by this tree, except *Pinus pumila*, which grows about the summit and is often found in the environment of the moor. *Taxus cuspidata* and *Tsuga diversifolia* are rarely found. The mixed forests of *Abies Mariesii* and *Fagus Sieboldi*, occupying the slopes below the coniferous forest region, are

[1] HORIKAWA, Y. 1930. The vegetation of Mt. Hakkôda. Sci. Rep. Tôhoku Imp. Univ. 4th Ser. (Biol.) Vol. 5 p. 555.

not remarkable in the neighbourhood of the moor. Dwarf trees of *Abies Mariesii* and *Pinus pumila*, together with shrubs, grow also in bushes found here and there on the moor[1]. Moreover, in the immediate neighbourhood of the moor, *Quercus crispula* and *Betula Ermanii* are found. *Betula Ermanii* with *Alnus Maximowiczii* and *Acer Tschonoskii* is often present in the higher part of this mountain. *Carpinus cordata* and *Ulmus laciniata*, as well as other deciduous trees, are only found in the lower parts far from this moor.

According to the results obtained from the pollen-analyses, *Abies* which is now dominant, seemed to be scarce in older times, and on the other hand, deciduous trees, especially *Fagus* and *Quercus* which are now rather seldom found in the neighbourhood of this moor, appeared to be predominant. On the basis of this fact, it may be presumed that deciduous forests have been invaded by the conifer, as is generally recognized in other regions.

SUMMARY.

As the result of pollen-analyses of peat layers formed on and between deposits of volcanic ash on Mt. Hakkôda, remarkable differences were recognized in the pollen composition in different layers.

Pollen of *Abies*, which were found in a larger number in the upper layer, could scarcely be seen in the lower layers, while those of *Fagus* and of *Quercus* were abundant in the lowest layer, decreasing remarkably towards the surface. This fact may be considered as an evidence of predominant growth of *Fagus* and *Quercus* in older times, while *Abies* is dominant in the present time. It may be presumed, therefore, that deciduous forests in this region have been invaded by the montane conifer.

I here wish to express my cordial thanks to Prof. Dr. Y. YOSHII for his kind suggestions, as well as for his kindness in providing the material and the photograph.

[1] Cf. YOSHII und HAYASI, l. c., p. 309.

On the Cardiac Nerves in the Oyster, *Ostrea circumpicta* PILS.[1]

BY

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(With 16 text-figures)

(Received Dec. 26, 1931)

INTRODUCTION.

The present experiment deals with the physiology of the cardio-regulator narves in the heart of the oyster.

There has been a controversy regarding the nervous control of the molluscan heart. As to the lamellibranchs, YUNG (1881) stated that the cardiac nerves of these molluscs (*Mya*, *Anadonta*, and *Solen*) have an acceleratory function, while, on the contrary, BUDDINGTON (1904) and CARLSON (1905) concluded that there is no evidence of the presence of the acceleratory nerves but the inhibitory nerves are alone present in the molluscs examined by them.

The purpose of the present experiment was to determine, if possible, whether or not the cardio-regulator nerves exist in the oyster, and if they do exist, to know the nature of the functions of the nerves.

This investigation was undertaken at the suggestion of Prof. Dr. S. HATAI at the Asamushi Marine Biological Station, and I wish here to express my hearty thanks for his kind guidance and encouragement, and also to the members of the station who have given valuable advice.

MATERIAL.

For the present work *Ostrea circumpicta* PILS. was used, since not only this oyster has been used for numerous other physiological experiments, but also it can be collected abundantly in the neighbourhood of the station.

The preparation of materials for the experimentation was proceeded in the following manner.

The shell was removed carefully so as not to injure the visceral ganglion

[1] Contributions from the Marine Biological Station, Asamushi, Aomori-Ken. No. 80.

and the pericardial region. The greater parts of the gills, together with the mantle, were cut away and the adductor muscle was then dissected along the dotted line in Fig. 1, then the oyster was fastened to a small board in such a manner that the heart and the visceral ganglion with its nervous connections are accessible for the stimulation (Fig. 2). The visceral ganglion lies on the ventral surface of the adductor muscle imbedded in a mass of connective tissue, and can be usually seen without any dissection through the tissue. The ganglion gives rise to, eight to nine pairs of nerves; *viz.*, cerebro-visceral connectives, the branchial nerves, the visceral nerves, the nerves to the adductor muscle, and several pairs of the nerves to the mantle. The connectives and the branchial nerves run parallel

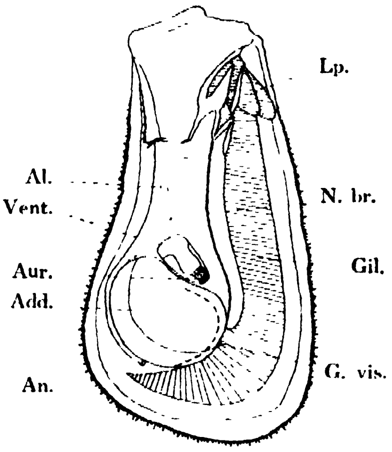

Fig. 1. Diagram to show the relative position of the visceral ganglion, the heart, the alimentary canal, etc Add, adductor muscle; Al, alimentary canal; An. anus; Aur. auricle; Gil, gills; G. vis, visceral ganglion; Lp, labial palps; N. br, branchial nerve; Vent, ventricle.

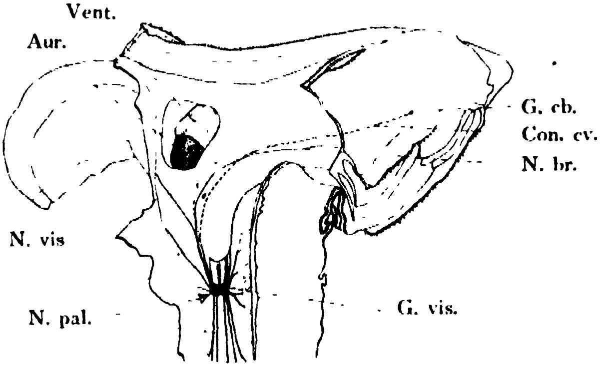

Fig. 2. Showing the nerves arising from the visceral ganglion. The adductor muscle was removed along the dotted line in Fig. 1, and the region of the visceral ganglion is turned upward. Aur, auricle; con. cv, cerebro-visceral connective; G. cb, cerebral ganglion; G. vis, visceral ganglion; N. br, branchial nerve; N. pal, pallial nerve; N. vis, visceral nerve; Vent, ventricle.

and close together for a distance of about one centimeter from the ganglion. The branchial nerves turn laterally and enter the gills, while the connectives penetrate the reproductive gland and liver, and then enter the cerebral ganglion at the ventral surface of the oesophagus. Close to the visceral ganglion each connective gives off a small nerve which runs parallel with it for a short distance and then turns laterally to enter the renal organ and the viscera. This nerve may be designated the visceral nerve, and one of the small branches of the nerve can be traced to the base of the auricle, but the nerve to the ventricle branches off so extensively in the renal organ that I was not able to trace any one branch of the nerve entering the ventricle directly.

EXPERIMENTS.

1. In the first series of the experiments I attempted to determine the effects of the stimulations of the visceral ganglion on the pulsations of the heart in *situ*. The apparatus deviced for this purpose is shown in Fig. 3.

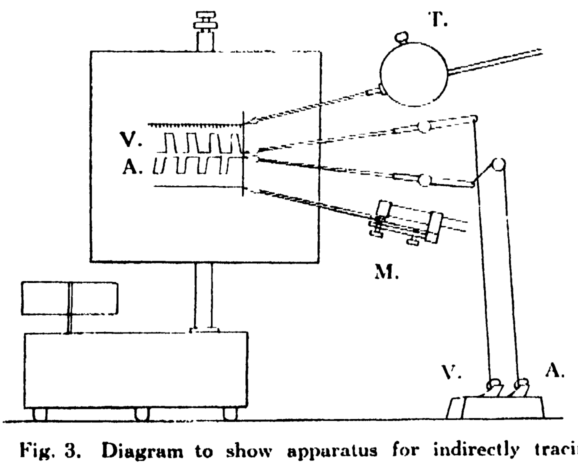

Fig. 3. Diagram to show apparatus for indirectly tracing the pulsations of the heart in *situ*. A, auricular rhythm; M, magnet to mark the instance of the stimulation; T, time mark; V, ventricular rhythm.

By means of this apparatus, the heart rhythm was recorded indirectly in the following manner. I pressed key A in Fig. 3 by hand at the beginning of the systole of the auricle and kept pressing till it was over and released the key at the beginnig of the diastole; and then in turn pressed

key V during the systole of the ventricle. The auricular and the ventricular rhythm were thus recorded. The small board to which the oyster was fixed in the manner as already stated was soaked in the sea water,

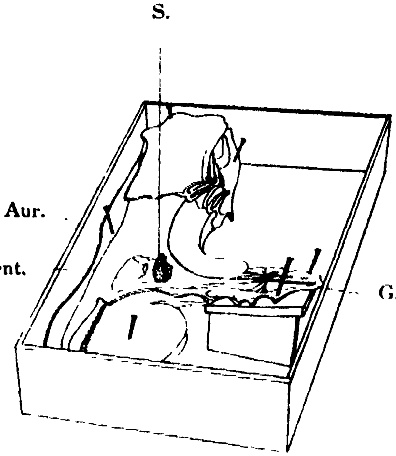

Fig. 4. Preparation in studying the effect on the auricular rhythm with the stimulation of the visceral ganglion. Aur, auricle; G. vis, visceral ganglion; S, silk thread connecting the auricles and the recording lever; Vent, ventricle.

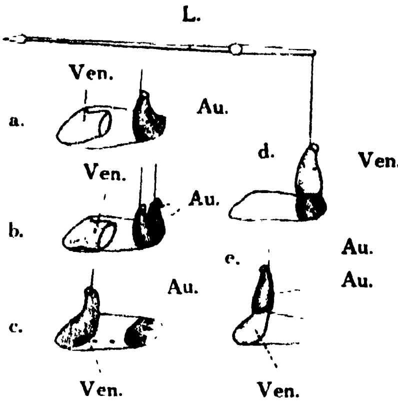

Fig. 5. Showing the manner of suspension. Au, auricle; Ven, ventricle; L, recording lever.

but the region of the visceral ganglion was exposed to the air. The pulsations thus recorded by the apparatus are shown in Fig. 6.

On stimulation of the visceral ganglion with a weak interrupted current, the pulsations cease in diastole, and complete arrest of the rhythm continues for half a minute but the beats usually reappear before the cessation of the stimulation (Fig. 6). The cardio-inhibitory mechanism of this heart

Fig. 6. The heart rhythm of the oyster is traced indirectly by means of the apparatus shown in Fig. 3. Showing the inhibition of the rhythm on stimulation of the visceral ganglion. The upper is the ventricular rhythm and the lower is the auricular rhythm.

shows fatigue or fails to respond very quickly when excited with a weak interrupted current. If the visceral ganglion is stimulated repeatedly, the threshold increases, but becomes ineffective if further stimulated.

Whether or not these reactions can be interpreted as a proof that the visceral ganglion sends inhibitory fibres to the heart can not be readily decided. In the oyster the base of the auricles is only about 3 cm. distant from the ganglion even in the largest specimens. It is, therefore, possible that the current may escape through the renal organ and stimulate the heart directly. The following experiments show that the inhibition was not caused by the escape of the current.

(i) If the heart is stimulated directly by such a strength of the stimulus as was applied to the visceral ganglion, the pulsations are not arrested.

(ii) If the interrupted current of the same strength is applied to the muscle adjacent to the visceral ganglion, the pulsations are not affected.

(iii) The inhibition of the pulsations is also obtained by the mechanical stimulation of the visceral ganglion. When the visceral nerve together with the cerebro-visceral connective is exposed and pinched with forceps at the point nearest to the ganglion, the pulsations are inhibited.

From the facts given above, we will be safe to conclude that the inhibition noted after an electrical stimulation was not caused by the escape of the current.

The visceral ganglion also sends fibers to the adductor muscle, the muscles of the mantle and the gills, but the present method of preparation cuts away the greater parts of these muscles, and the stimulation of the ganglion causes but only a slight contraction of the cut stumps of these parts. There is, therefore, no fear that a slight contraction of surrounding structures can modify the heart rhythm. I have, however, tested the effect of the contractions of these parts on the pulsations in the following ways.

(i) When the visceral nerve alone is exposed and stimulated mechanically as before, the pulsations are inhibited, but no contractions can be seen in these musculature.

(ii) If the visceral nerves are severed at the point nearest the ganglion, the stimulation of the ganglion produces no effect on the heart rhythm, although the contractions of the muscles of these parts are caused.

From these results, it can be concluded that the visceral ganglion sends inhibitory nerves to the heart.

II. With a view in determining the manner of innervation from the ganglion to the heart, the following experiments were performed.

1. The stimulation of the visceral ganglion on the auricular rhythm.

The preparation was made in the same manner as in the previous experiment (Fig. 2), and the auricles are cut at the auriculo-ventricular junction and the cut end is tied with a silk thread. The free end of the thread is connected to a recording lever and the rhythm was recorded (Fig. 4 and Fig. 5 a).

If now the visceral ganglion is stimulated with a weak interrupted current, the auricular rhythm is inhibited, but on cessation of the stimulation it starts at once to pulsate with a quicker rhythm than before for a several seconds (Fig. 7). The auricle responds very sensitively not only to the interrupted current but also to all other stimuli so far tested such as chemical and thermal either directly upon the ganglion, or by stimulation of the lateral and the posterior pallial nerves which arise from the ganglion. For instance, when the posterior pallial nerve is exposed carefully and stimulated with a weak interrupted current, the inhibition of the rhythm can be obtained.

Fig. 7. Showing the inhibition of the auricular rhythm on stimulation of the visceral ganglion.

From the results obtained after taking all precautions against the escape of the current and also avoiding any interference of the movement of the heart from undue pressure of adjacent parts, I am convinced to conclude that the inhibitory nerves enter the heart at the base of the auricles.

In the oyster heart, the right and the left auricular rhythms can be recorded separately (Fig. 5 b). The stimulation of the visceral ganglion arrests the rhythms of both auricles at the same time. The right and the left visceral nerves are exposed, and each nerve is severed at the point nearest the ganglion. By the mechanical stimulation of the left visceral nerve the rhythms of both auricles are inhibited (Fig. 8), and the stimulation of the right visceral nerve produces the same effect on both. It is therefore evident that each auricle is supplied with the inhibitory fiberes from both visceral nerves.

CARLSON (1905) concluded that there is no evidence of the presence of the acceleratory nerve in the lamellibranchs (*Mytilus*, *Tapes*, *Cardium*, *Pecten*, etc.), but in my present experiment I have obtained the results which may be considered to show the presence of the accelerator nerves.

Fig. 8. The right and left auricular rhythms are traced separately. Showing the inhibition of both rhythms on stimulation of the left visceral nerve.

In the normally pulsating auricles, as was shown already, the stimulation usually produces the inhibiting reaction. I have however encountered three peculiar preparations in which the accelerator effects were shown on the stimulation of the visceral ganglion. These preparations first showed the inhibitory effects several times repeatedly while the heart was pulsating

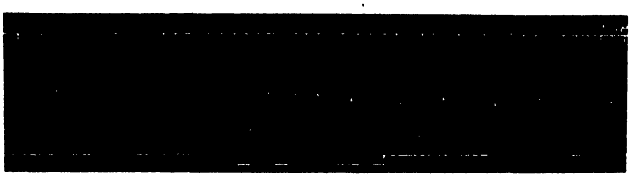

Fig. 9. The accelerator effect shown by the slowly beating auricle accompanied by stimulation of the visceral ganglion.

normally; but after keeping it in sea water for more than 30 hours and after the pulsations became slower, the stimulation of the ganglion produced acceleratory reaction decisively (Fig. 9). The results show that in the oyster both accelerator and inhibitory nerves are present in the heart, and that in normal condition an action of the inhibitory fibres predominates that of the accelerator when the two antagonistic nerves are stimulated simultaneously, or in other words the accelerator effects are overshadowed by the inhibitory effects. According to BOTAZZI and ENRIQUES (1901) and to CARLSON (1905) that in aplysia the inhibitory nerves are absent and the accelerator nerves alone are present. The presence of the accelerator

nerve may be easily demonstrated, if the cerebro-visceral connectives is stimulated with a weak interrupted current in a somewhat weakened aplysia. It must be remembered that the removal of the dorsal body wall, in order to expose the visceral ganglion usually causes so profuse bleeding that the heart is soon rendered empty and collapses (Fig. 10 and Fig. 11). On the other hand the stimulation of the visceral nerve on the aplysia having a vigorous and regularly beating heart usually produces inhibitory effects instead of acceleration (Fig. 12), and the complete arrest of the rhythm continues for some time. In this experiment, I have noticed the facts that the ventricle recovers pulsation with a remarkable augmentation when the same stimulus was applied to the nerve (Fig. 12.). These facts seem to suggest that which one of the antagonistic cardiac

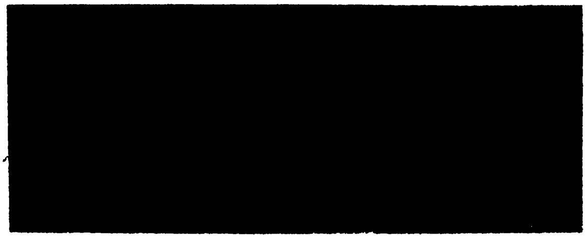

Fig. 10 Ventricular rhythm of *Aplysia*. Showing augmentation of the rhythm on stimulation of the cerebro-visceral connective.

Fig. 11. Quiescent ventricle of *Aplysia* begins and continues to pulsate during the stimulation of the cerebro-visceral connective.

Fig 12. Pulsating ventricle of *Aplysia*. Showing inhibition on the stimulation of the visceral nerve. The mechanical stimulation was applied to the visceral nerve.

nerves predominantly functions when stimulated, depends on the physiological conditions of the heart, but further investigations on this point are required.

2. The stimulation of the visceral ganglion on the ventricular rhythms.

The ventricle is severed at the auriculo-ventricular junction, and is connected to a recording lever by one end of a silk thread, the other end being tied to the cut end of the ventricle (Fig. 5 c).

On the stimulation of the visceral ganglion with a weak interrupted current, the ventricular rhythm is arrested (Fig. 13). The mechanical stimulation of the visceral nerve also produces the same effect. The same precautions were taken against the escape of the current and contractions of the adjacent parts as in the previous experiments.

Fig. 13. The ventricular rhythm of the oyster. Showing the inhibition of the rhythm on the stimulation of the visceral ganglion.

CARLSON stated that in the lamellibranchs the cardiac nerves enter the heart at the base of the auricles only and not at the aortic end. But in the oyster as is experimented here the cardiac nerves enter the heart also at the aortic end.

III. A series of experiments were carried out with the purpose of determining whether the cardiac nerves which enter the auricle at its base regulate not only the auricular rhythm but also the ventricular rhythm, and *vice versa*.

1. The aortic end is severed and the nervous connection through the ventricular end is intercepted; then the stimulation of the visceral ganglion has no appreciable effect on the ventricle; though its effect on the auricles is not diminished (Fig. 14). For kimographic registration the

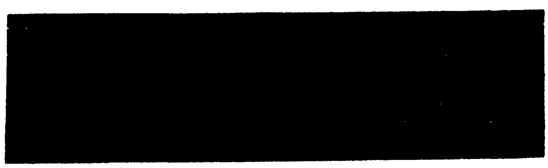

Fig. 14. Tracing of the heart rhythm recorded by the indirect method. The aortic end was severed. The stimulation of the ganglion fails to affect the ventricle when the aortic end was severed, though the auricular movement is inhibited.

ventricle was severed from the aortic end and a silk thread was secured at the cut end (Fig. 5 d). Now, if the ganglion is stimulated, the auricular rhythm only is arrested, but in some cases both rhythms are unaffected (Fig. 15). When the stimulation fails to affect both rhythms, I have removed the ventricle for the purpose of control, by severing at the auriculo-ventricular junction, and tested the behavior of the auricle only. It was then found that the auricular rhythm is arrested when the ganglion is

Fig. 15. Tracing from the heart suspended in the manner as shown in Fig. 5 d.

stimulated. From this fact, it may be considered that the failure of inhibiting both rhythms is not due to the faulty technique of the preparation of the nerves, but due to the fact that the cardiac nerves which regulate the auricular rhythm do not regulate the ventricular rhythm, and consequently the ventricle continues to pulsate, though the auricular rhythm is arrested by the stimulation of the nerves. Since the powerful contractions of the ventricle shadows the weaker contraction of the auricle, consequently the inhibition of the auricle when is attached with the former, becomes invisible.

From these experiments it may be concluded that the cardiac nerves enter the auricle at its base and regulate only the auricular rhythm and not the ventricular rhythm.

2. That the cardiac nerves which enter the ventricle at the aortic

Fig. 16. Tracing from the heart suspended in the manner as shown in Fig. 5 e.

end regulate only the ventricle and not the auricular rhythm, can be shown in the same way.

The auricle is separated from its base but left in connection with the ventricle, and connected to a recording lever by a silk thread (Fig. 5 e). The same results are obtained as in the last experiments, *viz.*, the stimulation of the visceral nerve arrests only the ventricular rhythm or fails to produce any effects on both rhythms (Fig. 16). It will be concluded from these results that the nerves which enter the ventricle regulate the ventricular rhythm only, and the nerves which enter the auricle at its base regulate the auricular rhythm only.

SUMMARY.

My experiments show that the heart of the oyster is supplied with the cardio-regulator fibers from the visceral ganglion. The inhibition of both auricular and ventricular rhythms is obtained on the stimulation of the visceral ganglion. The accelerator effect on the auricle appears in older preparations which showed a lowered vitality. It is, therefore, probable that both kinds of the cardiac nerves are present in the auricle, and that the action of the inhibitory nerve predominates that of the other and consequently when the both kinds of the nerves were stimulated simultaneously, the effect of the former nerve alone appears.

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Contributions to the Physiology of the Heart of Oyster.*
III. An Automaticity in the Ventricle and Auricle of the Heart as affected by the Temperature.

By

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(With 5 text-figs.)

(Received Dec. 28, 1931.)

INTRODUCTION.

It is a well known fact that in the higher animals, the physiological properties of the auricle and the ventricle of the heart differ from each other on the effects of the temperature, osmotic pressure and some drugs etc. But as far as I am aware, there are but only isolated investigations on this subject in lower animals such as mollusks. The present research was undertaken with the hope of determining the lower and higher limiting temperatures on the automaticity of the auricle and ventricle of the oyster.

MATERIAL AND METHODS.

For the materials in the present research, *Ostrea circumpicta* PILSBRY and *Ostrea gigas* THUMBERG were employed. The former species which attains considerable body size is widely distributed in Mutsu Bay and can be obtained in large numbers, while the latter species though which is also widely distributed in Japan, was collected at Takahokomura which faces the Pacific and is not very far from the Asamushi Marine Biological Station. *O. gigas* is not only somewhat smaller in size, but differs in many physiological behaviors from *O. circumpicta*.

In order to test the physiological properties of the two portions of the heart, two methods were utilized. One, is the complete removal of the heart from the body and then suspending both auricle and ventricle separately in the normal sea water and thus recording the pulsatory activities by the kymographic method. The second method is to record the activities by the similar kymographic method, by dividing and suspending

*Contributions from the Marine Biological Station Asamushi, Aomori-Ken No. 81.

separately the cut ends of the auricle and of the ventricle which were severed at the auriculo-ventricular junction while attaching the other intact ends to the body. In order to determine the effect of higher and lower temperatures on the pulsatory activities, the auricle and ventricle which were pulsating in sea water of about 200 cc capacity, were at first cooled with an ice and salt mixture and then heated gradually with an alcohol lamp. Recently WATANABE (1930) showed with various forms of Annelids that the heat shortening of the tissues are subject to considerable variation when the temperature was raised or lowered slowly or rapidly. The same holds true with the oysters not only for heat shortening, but also for the rhythmic contractions of the heart. Unfortunately, there is no method which completely obviates this difficulty, thus I was obliged to adjust the burner of the alcohol lamp so as to uniformly raise or lower the temperature.

A loaded weight will of course, affect the tonus, the rate of pulsation and shortening in tissues, and so I have chosen the lightest possible weight for this purpose.

EXPERIMENTAL RESULTS.

The auricle and ventricle which were suspended in the sea water pulsate automatically, though that of the ventricle is more vigorous than in the auricle. (Fig. 1).

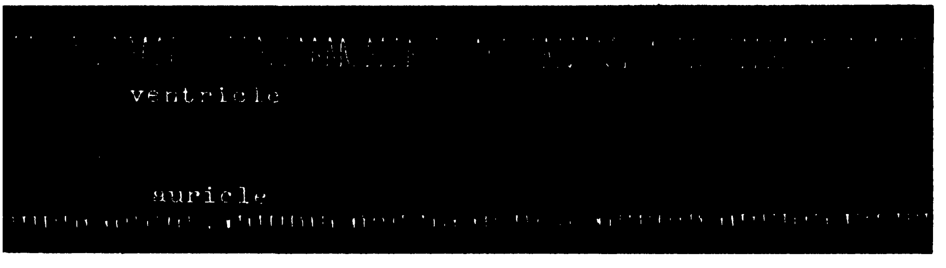

Fig. 1. Showing the pulsation of the ventricle and auricle recorded by the second method: that is dividing and suspending separately the end of the auricle and the ventricle at the auriculo-ventricular junction attaching the other ends to the body. (*O. gigas*).
Note the differences of the pulsatory power in the ventricle and auricle
The time marked per five minutes.
Temperature of the medium at 14.5°C.

The auricle and the ventricle behave differently in pulsatory activities as is seen from the following table. (Table I).

TABLE I.

Temp. at which the pulsation stops / No. Experiment	Ventricle				Auricle			
	A	B	C	D	A	B	C	D
1	38.5	7.0	7.0	45.0	43.0	3.0	5.0	47.5
2	42.0	6.0	15.0	44.0	43.5	2.0	7.0	47.0
3	37.5	5.5	7.5	45.0	42.5	6.0	5.0	46.0
4	45.0	8.0	7.0	42.0	44.5	4.0	6.0	47.0
5	38.0	6.0	8.0	45.0	45.0	3.0	7.0	47.5
6	37.0	8.0	7.0	45.0	42.0	2.5	6.0	47.0
7	38.5	7.5	6.5	46.0	40.0	1.5	5.0	47.5
8	37.5	5.5	5.5	44.0	43.0	3.0	6.0	47.5
9	38.5	6.0	5.0	47.0	41.5	2.0	7.0	48.0
10	37.0	5.5	15.5	43.0	46.0	3.0	6.0	47.0
11	38.0	4.0	8.0	45.5	42.0	4.5	6.0	47.5
12	37.5	6.0	10.5	42.0	45.5	5.5	5.5	49.0
Average	38.5	6.3	8.5	44.4	43.5	3.3	6.0	47.4

Table I. Showing the temperatures at which the pulsation stops in the ventricle and auricle. (*O. gigas*).

In A is shown the temperature °C at which the pulsation stoped.

In B is shown the temperature °C at which the pulsation stoped; gradually lowered from the room temperature.

In C is shown the temperature °C at which the pulsation begun; gradually raised from 0°C.

In D is shown the temperature at which the initial heat rigor occured.

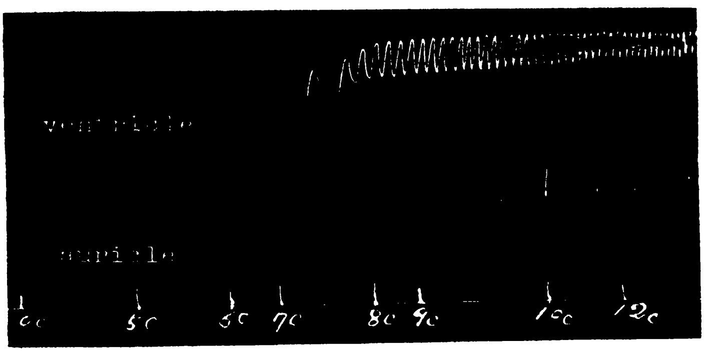

Fig. 2. No 1. *O. gigas* Showing the temperature C at which the pulsation began gradually upon being raised from about 0°C.

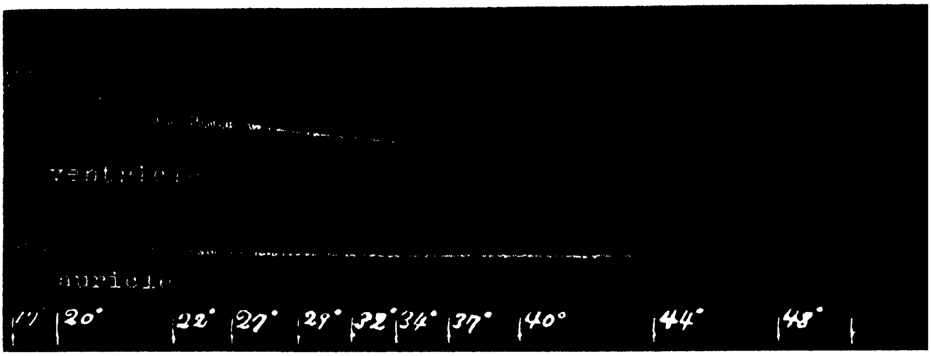

Fig. 2. No. 2. *O. gigas* Showing the temperature °C at which the pulsation stoped at a higher temperature and the temperature at which the initial heat rigor occured.

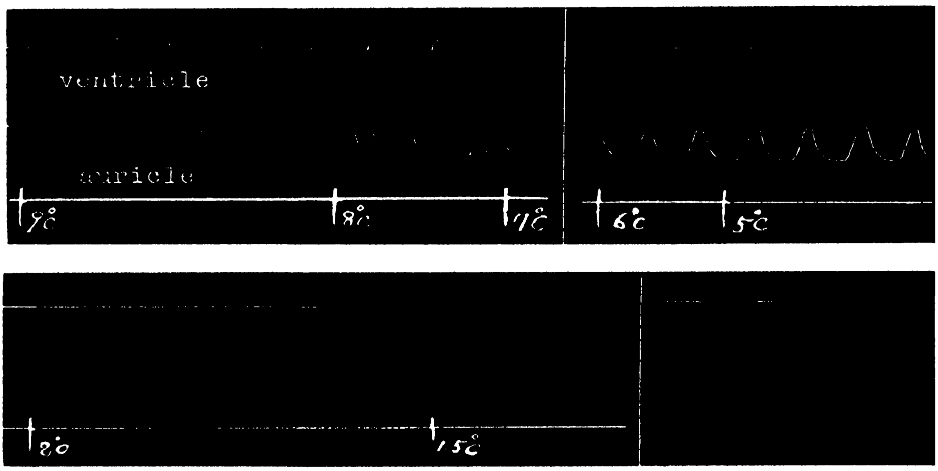

Fig. 2. No 3. *O. gigas* Showing the temperature °C at which the pulsation stoped gradually upon being lowered from the room temperature with an ice and salt mixture.

In Table I, the auricle shows not only a stronger resistance to both higher and lower temperatures than in the ventricle, but the former also differs from the latter in another physiological property as Table II and Fig. 3 show.

TABLE II.

Temperature °C	Tonus		Amplitude		Frequency	
	Ventricle	Auricle	Ventricle	Auricle	Ventricle	Auricle
0	1.0 cm	1 0 cm	0 cm	0 cm	0	0
5	1.0	1.0	0	0	0	0
6	1.0	1.2	0	0.4	0	3
7	1 0	1.0	0	0.5	0	4
8	1.2	1.2	0.5	0.5	3	5
10	1.6	1.4	0.7	0.6	6	6
13	2.0	1.5	0.8	0.7	8	8
15	2.0	1.5	1.2	1.2	10	12
20	1.7	1.0	1.1	1.0	14	14
25	1.4	0.8	0.9	0.9	20	18
30	1.3	0.8	0.8	0.8	25	24
35	1.3	0.8	0.7	0.5	30	32(37°C)
40	1.2	0.8	1.2	0.4	14	20
45	1.3	0.8	0	0.3	0	14
47	1.4	0.7	0	0	0	0
49	1.5	0.9	0	0	0	0
50	1.7	1.2	0	0	0	0
55	1.8	1.5	0	0	0	0

Table II. Showing some examples of the tonus, amplitudes and frequencies of the pulsations in the ventricle and auricle at varied temperatures (*O. gigas*).

Tonus....Height from base line in cm.

Amplitude ...Contraction height of pulsation in cm.

Frequency of pulsation ...The number of pulsations per minute

At a higher temperature, the resistance of the ventricle is somewhat different individually, but the averaged value for the complete inhibition of the pulsation is at 38.5°C. The pulsation of the ventricle at a lower temperature is feeble, but gradually increases its frequency as the temperature of the medium rises and gives the maximum frequency of pulsation of 30 per minute at about 35°C. On the other hand, if the temperature is raised above 35°C the frequency decreases and is completely abolished at about 40°C. The tonus of the ventricle shows the maximum at about 15°C but decreases steadily as the temperature rises till just before the onset of the initial heat rigor becomes evident. The amplitude of pulsation also reaches the maximum at about 15°C and becomes gradually smaller as the temperature becomes higher, though in some instance extraordinary vigorous pulsations are shown just before those were abolished. The initial heat rigor or primary heat shortening of the ventricle occurs in average at 44.4°C

HATAI (1924) and others, stated that the temperature necessary to produce the heat shortening of the tissues varies whether the materials

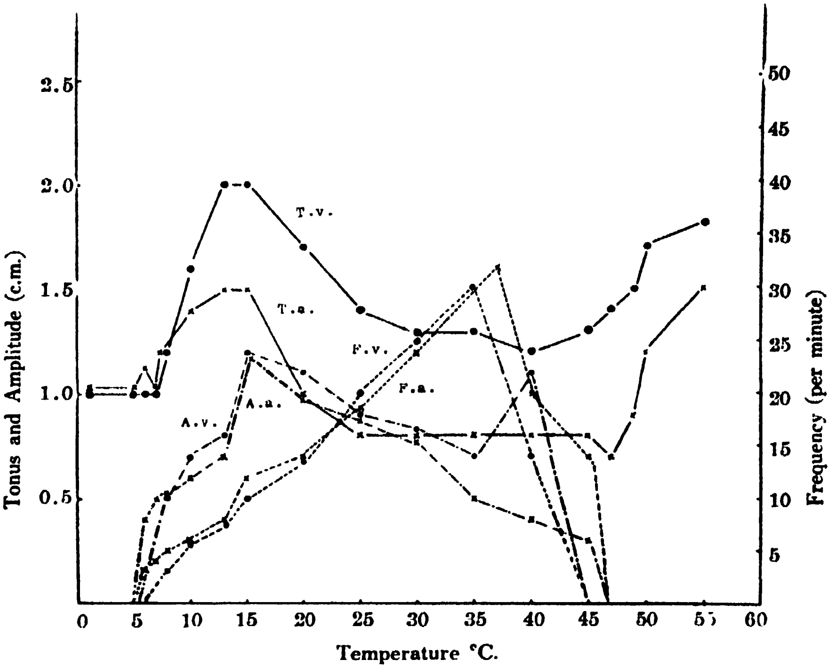

Fig. 3. Based on Table II in which the ordinate indicates the tonus, amplitude in cm. (left side) and frequency of the pulsation per minute. (right side).

The absissa indicates the temperature °C.

T. v. Tonus of ventricle
T. a. Tonus of auricle
A. v. Amplitude of ventricle
A. a. Amplitude of auricle
F. v. Frequency of ventricle
F. a. Frequency of auricle.

were fresh and active or not. This fact seems also true in the heart of oyster.

In the auricle, the general behaviors regarding the amplitude, the frequency of the pulsation and the tonus are essentially the same as those shown by the ventricle. The tonus and amplitude reach the maximum at about 15°C as with the ventricle. The maximum frequency is shown at about 37°C and is abolished at about 43.5°C. The initial heat rigor occurs at about 47.4°C indicating a slightly higher resistance than in the ventricle. Both in the ventricle and auricle the pulsatory power is

recovered if the temperature is lowered again before the onset of the initial heat rigor. (Fig. 4.)

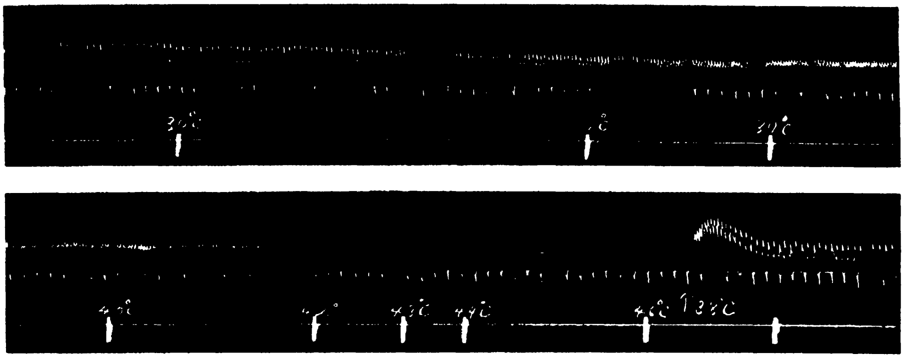

Fig. 4. *O. gigas* Showing the recovery of the pulsatory power of the temperature lowered again before the onset of the initial heat rigor. (auricle)

Note the sudden contraction of the auricle when the temperature was suddenly lowered.

The time is marked per 5 minutes.

In the lower temperature, the frequency as well as the amplitude of the ventricular pulsation not only decreases gradually but becomes irregular. The pulsation of the ventricle is abolished at about 6.3 C. The auricle also shows gradual decrease of the pulsation on lower temperature but exhibits stronger resistance than in the ventricle. Indeed it is abolished at 3.3°C and in some individuals the pulsations are seen even at 1.5 C. The amplitude as well as the tonus decreases gradually in both auricle and ventricle when these are exposed into the lower temperature.

In both the auricle and ventricle, the pulsation is completely stoped at about 0°C. If however, the ventricle and auricle at about 0°C are again heated gradually with the alcohol lamp a recovery process somewhat differs in those two portions of the heart. The auricle begins to pulsate at a much lower temperature than in the ventricle, that is the auricle begins to pulsate at about 6.0 C while that of the ventricle at about 8.5 C. (average) The temperature at which the tonus of the ventricle and of the auricle increases rapidly and begins to pulsate is not identical whether the temperature was raised from 0°C or whether lowered towards 0°C. The cause of this phenomena just mentioned is not entirely clear, but it may partially be due to the difference in the colloidal state of the cardiac muscle whether it was first exposed to the higher or lower temperatures.

The work done by the heart of oyster during its pulsation has been calculated and was reported already by the present author (1927), and I have undertaken in the present research, similar calculations separately on the auricle and ventricle. The work done by the ventricle and auricle at varied temperatures is calculated by the following simple formula;

Number of pulsations per minute × Amplitude of the contraction × Tonus (The hight from the base line) = Work done.

TABLE III.

Temperature	Ventricle	Auricle	Ventricle & Auricle
0	1	1	2
5	1	1	2
6	1	1.44	2.44
7	1	2.00	3.00
8	1.80	3.00	4.80
10	6.72	5.04	11.76
13	12.80	8.40	21.20
15	24.00	21.60	45.60
20	26.18	14.06	40.18
25	25.20	12.96	38.16
30	26.00	15.36	41.36
35	27.30	12.00	39.30
40	20.16	6.40	26.56
45	1.30	3.36	4.66
47	1.40	0.70	2.10
49	1.50	0.90	2.40
50	1.70	1.20	2.90
55	1.80	1.50	3.30

Table III. Showing the work done by the ventricle and auricle at varied temperature. The absolute work done by the auricle and ventricle is based on the Table II.

As will be seen from Table III and Fig. 5, the work done by the ventricle rapidly increases from 10°C up to 15°C and reaches the maximum at 35°C. After 35°C it drops very rapidly and reaches the minimum at 45°C. The auricle on the other hand, performs the maximum work at 15°C which is followed by a decrease and reaches the minimum at 47 C, as in the case of the ventricle. The work done by these two portions of the heart differs from each other under the same conditions and that of the ventricle exceeds far greater the auricle. These differences just stated may be due to the differences of the physiological and anatomical properties of the tissues concerned; that is, the ventricle is more muscular than in the auricle and since the former being the chief center of the output of the blood, hence produces greater work than in the auricle.

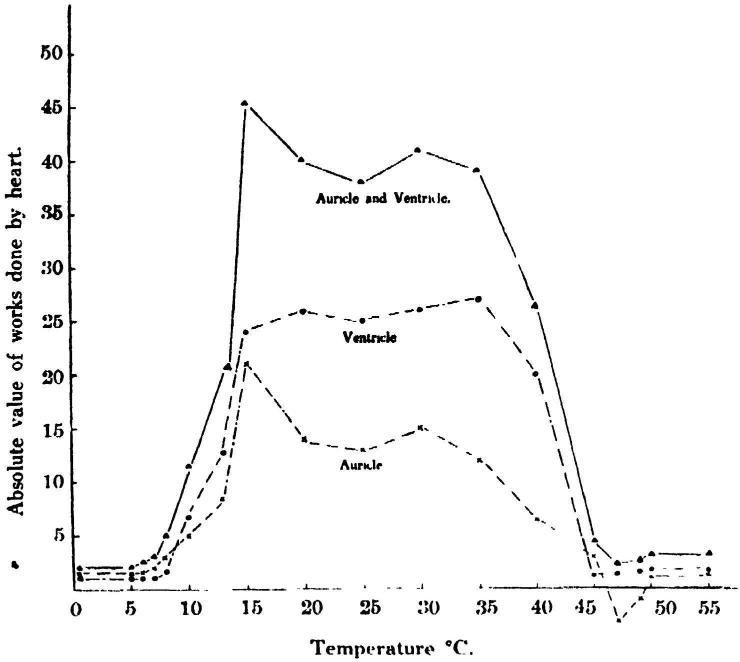

Fig. 5. Based on the Table III in which the ordinate indicates the absolute value of the work done by the heat and the absissa of the temperature °C. (*O. gigas*)

—•—·—•—·— Ventricle
·×—·—×—·— Auricle
——▲——▲— - Ventricle and auricle.

GENERAL CONSIDERATIONS.

GELLHORN (1924) found that the heart strip of the frog immersed in RINGER's solution, produces the two types of the effects when acted by the heat; that is, the amplitude of the pulsation does not clearly decrease at the higher temperature, but the frequency of the pulsation increases and consequently the work done also increases. He considered that this type is a physiological one. In another type, the amplitude gradually decreases followed by an increased temperature but the frequency of the pulsation is increased and consequently the work done decreases. FUNADA (1928) stated that these two opposite phenomena observed by GELLHORN are mainly due to whether the given heart strips possessed in it many or a few number of the nervous ganglion cells, and thus he concluded that

the first type indicates the presence of many ganglion cells while the second type indicates fewer ganglion cells in the strips. These two types of the reactions can not be clearly demonstrated in the oyster heart. The innervation of the regulative nerves of the heart pulsation has been studied by OKA (1932) physiologically, but as the presence or absence of the ganglion cells in the heart was not stated by him and it seems, therefore, highly important to study the distribution of the nervous ganglion cells in order to scrutinize the possible relationship of the nerve cells to the activity of the oyster heart, in the future.

The physical changes which occur in protoplasm as the temperature is either raised or lowered many throw light on some of the fundamental properties of the pulsation of the heart. As for instance, BĚLĚHRADEK (1928) stated that the cardiac frequency of the *Daphnia* is slowed in the lower temperature due to increased viscosity of the protoplasm, and that its complete stoppage, is associated with a reversible gel formation.

HEILBRUNN (1926) who measured the viscosity of the egg plasm under various temperatures found that in the lower temperature at 1° above freezing point the viscosity increases suddenly and again in a high temperature at 31°C. it also increased suddenly. The maximun viscosity in eggs is at 15°C. These facts just stated may very likely be the phenomena similar as with the phenomena of the heart pulsation, since as I have already stated, the rapid increase of the amplitude occurs just before the pulsation stops at a high temperature.

The effect of the temperature on the viscosity of protoplasm has been studied by several other investigators (WEBER 1923, PANTIN 1924, BECKING 1928, etc.) though the results attained by them are not entirely consistent, however, it seems probable that the pulsation, amplitude and tonus of the heart which either decreases or increases at lower or higher temperatures may be associated with the gelation of its protoplasm as will be seen from the occurrence of the optimum condition attained at about 15°C. in the heart of oyster.

According to HEILBRUNN (1928) the heat coagulation of protoplasm depends primarily on an alteration of the fats or lipoids of the cell, and therefore, it is easy to understand that an increase in water content would favor heat death and a decrease in water concentration would retard it. Since the higher the percentage of water in the cell, the more readily would fat and lipoid tend to dissolve. If this view of HEILBRUNN were true, the protoplasm of the ventricle would contain a greater amount of water than in the auricle, but such an assumption needs further

verification. At any rate the heat rigor of the living cell must be due to the many factors as mentioned above and by the differences in their interactions producing the difference of activity in tissues and organs when affected by either lower and higher temperature accordingly.

CONCLUSION.

The auricle is much stronger in its resistance than in the ventricle when exposed to lower and higher temperature; that is, in the ventricle, the pulsation is completely inhibited at about 6.3°C. and 38.5°C while in the auricle it is inhibited at about 3.3°C and 43.5°C. The initial heat rigor occurs at about 44.4°C. in the ventricle and at about 47.4°C. in the auricle. The tonus of the ventricle and auricle is the maximum at about 15°C. The amplitudes of the ventricle and the auricle show the maximum at about 15°C. The frequencies show some difference in the two portions; that is, in the ventricle, it is maximum at about 35°C. and in the auricle at about 37°C. These differences are assumed as due to the difference of the colloidal behavior of protoplasm which constitutes it and reasons for it are presented in the text.

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Studien über die Bildung organischer Säuren in grünen Pflanzen. I.

Die Reihenfolge des Säuregehalts im ganzen Körper von *Begonia Evansiana* ANDR.

VON

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(Mit 6 Textfiguren)

(Eingegangen am 8. Jan. 1932)

I. EINLEITUNG UND ÜBERSICHT ÜBER DIE FRAGE DER OXALSÄURE IN GRÜNEN PFLANZEN.

Die Oxalsäure ist nicht nur ein verbreitetes, sondern auch ein sehr auffälliges Produkt des Stoffwechsels der Pflanzen, da sie in der Regel in Form von Kristallen ihres Kalksalzes vorkommt. Kalkoxalat findet sich, mit Ausnahme der Lebermoose, in allen Pflanzen, denn LORCHE hat sein Vorkommen auch bei Laubmoosen festgestellt, die früher immer für kalkfrei gehalten wurden. Nach BRACONNOT findet es sich in enormen Massen in einigen Flechten: z. B. in *Pertusaria communis* zu 17%, in *Chlorangium Jusufii* zu über 65% der Trockensubstanz (ausserdem auch lösliches Oxalat).

Überdies findet sich die Oxalsäure als freie Säure und Mg-, K- und Na-Salze in der Pflanzenwelt. Nach MONTEVERDE tritt das Mg-Oxalat bei zahlreichen Paniceenarten in Form stark doppelbrechender, radialstreifiger Sphäriten, oder in Form unregelmässiger Aggregate fast in jeder Zelle der Epidermis trockner Blätter, seltner in ihren Mesophyllzellen auf. Als saures Kaliumsalz, im Zellsafte gelöst, findet sie sich in vielen *Oxalis*- und *Rumex*arten, in Blättern von *Rheum*, *Geranium* und vielen anderen Pflanzen und als lösliches Natriumsalz in *Salicornia* und *Salsola*.

Wegen dieser grossen Verbreitung der Oxalsäure in der Pflanzenwelt wurde man schon frühzeitig auf diese Säure in physiologischer Hinsicht aufmerksam. Trotz zahlreicher Arbeiten verschiedener Forscher bleibt aber bis heute die Bildungsweise und Bedeutung dieser Säure noch etwas ungeklärt. Daran trägt im grossen und ganzen die bisher verwendete Untersuchungsmethode Schuld.

Bevor wir aber auf die Besprechung unserer eigenen Versuche eingehen, so sei zunächst der gegenwärtige Stand der Kenntnisse von dieser Frage gegeben. Folgende vier Punkte kommen hier in Betracht: a. Durch welche chemischen Vorgänge entsteht sie in der Pflanze? b. Welche Rolle spielt sie im Leben der Pflanze? c. Welche Bedingungen beschleunigen oder verzögern die Oxalsäurebildung? d. Ist die Verarbeitung der Oxalsäure enzymatischer Natur?

a) Die Frage der chemischen Vorgänge bei der Oxalsäureentstehung.

Was zunächst die Bildungsweise der Oxalsäure anbetrifft, so hat schon 1840 LIEBIG berichtet, dass die Oxalsäure, wie die anderen organischen Säuren, als Zwischenprodukt bei der Reduktion und Kondensation der Kohlensäure durch den Chlorophyllapparat der höheren grünen Pflanzen entsteht. Dieser Auffassung entstanden meisten Pflanzenphysiologen der neueren Zeit Gegner, weil erstens die Oxalsäurebildung in den grünen Pflanzen mit Oxydationsvorgängen und nicht mit Reduktionsvorgängen zusammenhängt, weil sich zweitens die Empfindlichkeit des Chlorophylls gegenüber der sauren Reaktion geltend macht, und weil drittens die Bildung eines Kohlenhydrats aus Oxalsäure vom chemischen Standpunkt aus nicht begreiflich ist, um so mehr als die Umwandlung von Kohlensäure in Oxalsäure nur mit geringer Erhöhung des energetischen Potentials verbunden ist.

Obgleich die LIEBIGsche Hypothese fast allgemein als nicht zutreffend aufgegeben wurde, so wurde sie doch noch von Zeit zu Zeit von verschiedenen Seiten gestützt. BALLO, der aus Ameisensäure Oxalsäure mittels Salpetersäure erhielt, sah es für möglich an, dass die Aufeinanderfolge der Reduktion so verläuft: Oxalsäure — Tartronsäure — Weinsäure — Aposorbinsäure — Zuckersäure usw. Eine ähnliche Bildung kohlenstoffreicherer Säuren aus einfacheren bei Pflanzen ist auch von BRUNNER und CHUARD und von KÖNIGS angegeben worden. TRAUBE unterstützte diese letzte Ansicht, indem er durch Reduktion des Oxalesters Glykolsäure, Apfelsäure usw. „in einfache genetische Beziehung zur Oxalsäure und damit zur Kohlensäure" brachte. Vor kurzem hat BAUR die LIEBIGsche Hypothese wieder aufgenommen und auf Grund energetischer Berechnung die Möglichkeit einer primären Reduktion von Kohlensäure zu Oxalsäure erörtert.

PALLADIN suchte zu beweisen, dass die organischen Säuren im wachsenden Pflanzenteile als Nebenprodukt bei der Regeneration des Eiweissstoffs aus Asparagin und Kohlenhydraten entstehen. SCHIMPER dachte gleichfalls, die Oxalsäure sei ein Nebenprodukt bei der Eiweissbildung, und HOLZNER fasste die Oxalsäure auch als Produkt der Proteinstoffe auf.

ARTHUR MEYER hat ähnlich die Bildung des Kalkoxalats mit der Eiweiss- und Säurebildung in Beziehung gebracht und festgestellt, dass die drei Prozesse — Eiweissbildung, Abnahme der freien Oxalsäure und Kalkoxalatbildung — miteinander korrespondieren. Bei kräftiger Eiweissbildung in beleuchteten Laubblättern erfolgt dementsprechend stets energische Abnahme der freien Oxalsäure und reichere Oxalatbildung. In letzter Zeit sind viele Versuche über die organische Säurebildung in den Pflanzen ausgeführt worden, und viele wertvolle Arbeiten brachten bedeutungsvolle Vorschläge in dieser Richtung. Besonders RUHLAND und seine Schüler benutzten als Versuchsobjekte höhere grüne Pflanzen im Gegensatz zum Pilz, der bisher häufig verwendet wurde. Nach der Untersuchung des Säurestoffwechsels von *Begonia semperflorens* kamen sie zum Schluss, dass das Auftreten der Oxalsäure aufs engste mit dem Eiweissstoffwechsel verknüpft ist. Bei *Begonia* besteht innige Wechselbeziehung zwischen dem Stickstoffwechsel und dem Auftreten der Säure. Nach WETZEL gibt es drei Möglichkeiten für die Oxalsäurebildung in den Pflanzenzellen, 1. durch Zuckeroxydation, 2. durch Desaminierung von Aminosäuren und Säureamiden und 3. durch Eiweisssynthese.

Auf die Möglichkeit der Oxalsäurebildung durch Zuckeroxydation wurde seit alten Zeiten von vielen Forschern hingewiesen, und von CZAPEK wurde in seinem trefflichen Buch „Biochemie der Pflanzen" die Oxalsäure als Produkt unvollständiger Oxydation unter gleichzeitiger Spaltung des Zuckermoleküls bezeichnet. Wenn aber diese Ansicht richtig wäre, so müsste man erwarten, dass säurereiche Pflanzen einen sehr niedrigen Haushalt hätten und als Folge des Kampfs ums Dasein zu Grunde gehen müssten. In Wirklichkeit gedeihen sie trotzdem überall auf der Erde und unterdrücken sogar andere Pflanzen. Schon allein diese Tatsache genügt, zu beweisen, dass die Zuckeroxydation wenigstens nicht der Hauptfaktor bei der Säurebildung ist. Die Möglichkeit der Säurebildung durch Eiweisssynthese und durch Desaminierung von Aminosäuren und Säureamiden scheint viel wahrscheinlicher als die durch Zuckeroxydation.

Nach RUHLAND und seinen Schülern geht ja das Auftreten der organischen Säuren der Desaminierung parallel, und der dabei gebildete stickstofffreie Restkörper kann die Muttersubstanz der organischen Säuren sein.

b) Die Bedeutung der Oxalsäure im Leben der Pflanzen.

Über die physiologische Bedeutung dieser Säure meinte DE VRIES, im allgemeinen seien die Oxalate als Exkret anzusehen, sie seien aber nicht unbedingt nutzlos, da sie als Turgorstoffe der Zelle dienen können. GR. KRAUS meinte aber auf Grund seiner quantitativen Versuche, das Kalk-

oxalat der Baumrinden als Reservestoff hinstellen zu sollen. Nach HOLZNER und SACHS sollen die organischen Säuren, insbesondere Oxalsäure, den Zweck haben, aus Kalziumphosphat und Kalziumsulfat die Säuren frei zu machen und so den Pflanzen die Assimilation der Phosphor- und Schwefelsäure zu ermöglichen; aber diese Hypothese hat heute nur noch historisches Interesse.

Andererseits sieht SCHIMPER die giftige Oxalsäure in erster Linie als Nebenprodukt des Stoffwechsels an. Für die Pflanze ist es deshalb notwendig, sie unschädlich zu machen oder mindestens aus dem Stoffwechsel auszuschalten. Der Kalk erfüllt seine Aufgabe zwar mit dem Augenblick, wo er mit der Oxalsäure zusammentrifft und als Kalkoxalat verbunden wird. DE VRIES, PFEFFER und WEHMER vertreten nun gerade die entgegengesetzte Auffassung. Nach ihnen übt der im Überschuss aufgenommene Kalk, sei es als Kalzium-nitrat, -sulfat oder -phosphat, eine schädliche Wirkung auf den Organismus aus, die durch Bildung von Oxalsäure und daraus folgende Fällung von Kalkoxalat beseitigt wird. Nach SCHLEIDEN und FRANK ist die Bedeutung der organischen Säuren ebenfalls in der Neutralisation der überschüssig aufgenommenen Basen zu suchen. RUHLAND und WETZEL kamen zum Schluss, dass bei *Begonia semperflorens* die organischen Säuren als Entgifter des bei der Desaminierung zugleich auftretenden Ammoniaks eine Rolle spielen.

Vor kurzem beschäftigte sich NIETHAMMER mit der Bio- und Histochemie der Früchte und Samen und berichtete, dass man in unreifen Früchten und Samen oft Kalkoxalatausscheidungen findet, die mit zunehmender Reife meistens verschwinden, und dass die Kalkoxalatdrüsen demnach wieder im Stoffwechsel verwendet werden und daher keine nutzlose Auswurfstoffe sein können und dass in manchen Früchten bestimmter Familien im Zustand der Überreife Inklusen auftreten.

Ohne hier auf die ökologische Anschauung STAHLS oder auf den an die Reizperzeption anknüpfenden Gedanken HABERLANDTS näher einzugehen, so ist es doch verständlich, dass die physiologische Rolle der Oxalsäure seitens der verschiedenen Autoren ja nach den verschiedenen Versuchsobjekten ganz anders aufgefasst werden musste.

c) *Die bei der Säurebildung in Frage kommenden Bedingungen.*

Licht. Als völlig sichergestellt sind vor allem folgende Tatsachen anzusehen, erstens Abnahme der freien Säure der Fettpflanzen bei Tageslicht, zweitens Säurezunahme gleich nach der Verdunkelung und drittens schnelle Abnahme bei gleichzeitiger Erwärmung der Pflanzen während der Verdunkelung (A. MAYER, DE VRIES, GR. KRAUS). WARBURG glaubt, die

Lichtwirkung beruhe auf drei verschiedenen Vorgängen: der Lichtentsäuerung, der Dunkelentsäuerung und der Ansäuerung nach vorhergegangener Belichtung. Nach DE VRIES gibt es aber nur zwei Prozesse: erstens Entsäuerung, die beständig vor sich geht, und zweitens Ansäuerung nach vorhergegangener Belichtung, die dann jenen Prozess verdeckt. Also erfolgen in den Fettpflanzen beide Vorgänge der An- und Entsäuerung beständig nebeneinander, und je nachdem der eine oder der andere überwiegt, zeigen sich die Ergebnisse als Zu- oder Abnahme der Säure, während bei den eigentlich dünnblättrigen Pflanzen keine Unterschiede bei Tageslicht nachzuweisen sind.

In den Studien über den Säurestoffwechsel sukkulenter Pflanzen mittels einer neuen Methodik zeigte aber BENDRAT, dass bei alten und mittleren Blättern während der Nacht tatsächlich Ansäuerung erfolgt, bei jungen Blättern aber, im Gegensatz dazu, nachts wiederholt Entsäuerung. Die Entsäuerung setzt bei alten und mittleren Blättern in den ersten Tagesstunden schwach ein und findet in der Hauptsache in der Mittagszeit statt. In den Nachmittagsstunden kann, besonders bei jungen Blättern, bereits wieder Ansäuerung eintreten. Die Säureschwankungen sind also nicht nur von äusseren, sondern ebenso stark von inneren Faktoren abhängig, aber die ausgesprochen qualitativen und quantitativen Unterschiede des täglichen Säurestoffwechsels verschiedener Blätter zeigen sich nicht in jahreszeitlichen Schwankungen. SCHIMPER meinte, dass das Licht ohne Einfluss auf die Bildung des Kalkoxalats in chlorophyllfreien Blattteilen ist. Die Bildung des sog. sekundären Kalkoxalats ist demnach zwar abhängig von Licht und Chlorophyll, aber nicht von der Assimilation.

SPOEHR hat nun festgestellt, dass in Quarzgefässen der Sonne oder dem Quecksilberdampflicht ausgesetzte wässerige Lösung organischer Säuren langsam zerfällt. Im Destillat einer belichteten wässerigen Apfelsäurelösung fand er neben CO_2 Ameisen-, Essig-, Glykol- und Oxalsäure, ferner Acet- und Formaldehyd. INGHILLERI hat ebenfalls durch Belichtung von Formaldehyd und Oxalsäure unter Mitwirkung eines Katalysators eine Monose erhalten.

Temperatur. 1875 wies A. MEYER darauf hin, dass niedrige Temperaturen Vermehrung des Oxalsäuregehalts in den Pflanzen hervorriefen. Nach mehreren Forschern (DE VRIES, KRAUS usw.) tritt, wie oben erwähnt, während der Verdunkelung schnelle Säureabnahme bei gleichzeitiger Erwärmung ein.

Stickstoff. BENECKE hat die von WEHMER an Pilzen gemachte Erfahrung an grünen Pflanzen bestätigt, indem er die Pflanze zwang, ihren

die an diese Fragen mit der neueren Mikromethode herangehen. Als Versuchsobjekte höhere grüne Pflanzen wählend, gebe ich die Ergebnisse unter einem gemeinsamen Titel in einer Reihe wieder. In dieser ersten Mitteilung wird zunächst die Reihenfolge des Säuregehalts im ganzen Körper von *Begonia Evansiana* ANDR. behandelt.

II. MATERIAL UND KULTURMETHODE.

Als Versuchspflanze diente mir *Begonia Evansiana* ANDR., in der fast ausschliesslich Oxalsäure gebildet wird. In den folgenden Versuchen wurden aber Bulbillen als Ausgangsstadium zur künstlichen Kultur benutzt, weil durch Vorversuch sowohl an Knollen als auch an Bulbillen, gleichgültig ob sie jungen und noch grün, oder schon alt und schwarzbraun waren, keine Spur von Oxalsäure nachzuweisen war. In den übrigen Pflanzenteilen, wie Blättern, Stengel, Blüten, und Kelchblättern usw., wurde sie dagegen deutlich nachgewiesen. Um diese Säure nachzuweisen, wurden Sr- und Ca-Nitratlösung als Reagenz, oder die Mikrosublimationsmethode verwendet, worüber später noch näher gesprochen werden wird.

Nach der Reifung wurden Bulbillen, die schon Ende Herbst schwarzbraun werden, gesammelt und gesiebt, um möglichst gleichmässig grosse zu bekommen; in feuchten Quarzsand gesät, der vorher gut mit Salzsäure behandelt und danach mehrmals mit destill. Wasser gewaschen war, dann im Eisschrank gehalten, dessen Temperatur stets etwa 10°C betrug. Im folgenden Jahre wurden die Bulbillen je nach Bedürfnis aus dem Eisschrank genommen und in Wasserkultur gesetzt. Als Kulturgefäss diente hauptsächlich die sog. Alkaliflasche mit etwa 400 ccm Inhalt. Die Gefässe wurden zunächst in Chromschwefelsäurelösung über Nacht getaucht und nachher mit Wasser gut gewaschen. Die Aussenseite der Gefässe wurde zuerst mit schwarzer Emaillefarbe, die das Wurzelsystem vor Lichtzutritt schützt, und dann mit weisser bestrichen, um die Absorption der Sonnenstrahlenwärme zu verhindern. Im allgemeinen wurde in jedes Gefäss je eine Kultur gepflanzt. Meistens standen die Kulturen im Gewächshaus in möglichst heller Beleuchtung. Alle Versuchpflanzen wurden in 0.1%iger KNOPscher Nährlösung (ohne KCl) gezüchtet: die Lösung wurde etwa alle ein oder zwei Wochen erneuert, damit die Pflanzen während der ganzen Versuchsdauer eine ziemlich gleichmässige Zufuhr von Nährlösung geniessen konnten. In dieser Nährlösung gedieh *Begonia Evansiana* sehr gut, und zwar bis zum Ansetzen der Blüten.

III. SÄUREBESTIMMUNGSMETHODE.

Vor kurzem untersuchten RUHLAND und seine Mitarbeiter den Säurestoffwechsel von *Begonia semperflorens* und *Rheum hybridum Hort.* u. a. nach einem neuen genauen analytischen Verfahren, das aber bis heute noch nicht veröffentlicht worden ist. Nach langen Vorversuchen, die in Begonien frei oder gebunden vorkommende Oxalsäure zu bestimmen, fand ich die Mikrosublimationsmethode als für meine Zwecke am geeignetsten. Mit dieser Methode gelang es schon KLEIN und WERNER freie und gebundene Oxal-, Bernstein-, Apfel-, Wein-, und Zitronensäure im pflanzlichen Gewebe voneinander zu trennen und qualitativ nachzuweisen. Sie unternahmen auch den Versuch, die Methodik auf gravimetrischem Wege quantitativ auszubauen, worüber wir aber bis jetzt noch nichts Näheres gehört haben. Für unseren Zweck wurde der Apparat zur genannten Sublimationsmethode etwas verbessert, was uns veranlasste, die Oxalsäure quantitativ wieder zu finden. Bei dieser Methode zersetzt sich die Oxalsäure nicht vollständig.

Die Sublimate bestehen in der Regel am Anfang der Sublimation aus längeren Prismen, die bald in kleinere Kriställchen zerfallen. Wenn das Material nicht so gross war, betrug die Ausbeute ca. 99%. (Tabelle 1).

TABELLE 1.

Reine Oxalsäure.

Nummer des Schälchens	Gewicht der Oxalsäure (g)	Gefundene Oxalsäure (g)	Prozent
1	0.000758	0.000756	99.73
2	0.001297	0.001287	99.22
3	0.001830	0.001813	99.07
4	0.002291	0.002259	98.61
5	0.002301	0.002261	98.26
6	0.002469	0.002413	97.73

A. Apparat.

Der Sublimationsapparat aus hartem Glas besteht aus einem Mantel (M), einem mit Thermometer versehenen Tubus (T) und einseitiger Tubulierung zum Evakuieren (G), und einem Kühler (K), der in den konischen Hals des Mantels vakuumdicht eingeschliffen ist. Im Kühler sind zwei Glasröhrchen eingeschmolzen, von denen das eine, bis zum Boden des Kühlers reichende, zur Wasserzuleitung (a), das kürzere zur -ableitung (b) dient.

Um die Geschwindigkeit des Wasserstroms von aussen her leicht schätzen zu können, wurden zwei oder drei Gummistückchen auf den Boden des Kühlers gelegt. Die geschliffenen Teile des Kühlers sowie der konische Hals des Mantels wurden mit dem pulversierten Graphit eines Bleistifts (BBB oder BBBB ist geeignet) vakuumdicht gehalten. Vaselin oder wasserfreies Lanolin sind für diesen Zweck nicht so geeignet wie der Graphit des Bleistifts, weil beide bei Wärme schmelzen und beim Herausnehmen des Kühlers leicht am Rand des Deckglases, das zur Aufnahme der Sublimate auf der äusseren Seite des ebenen Bodens des Kühlers festgemacht wurde, kleben bleiben.

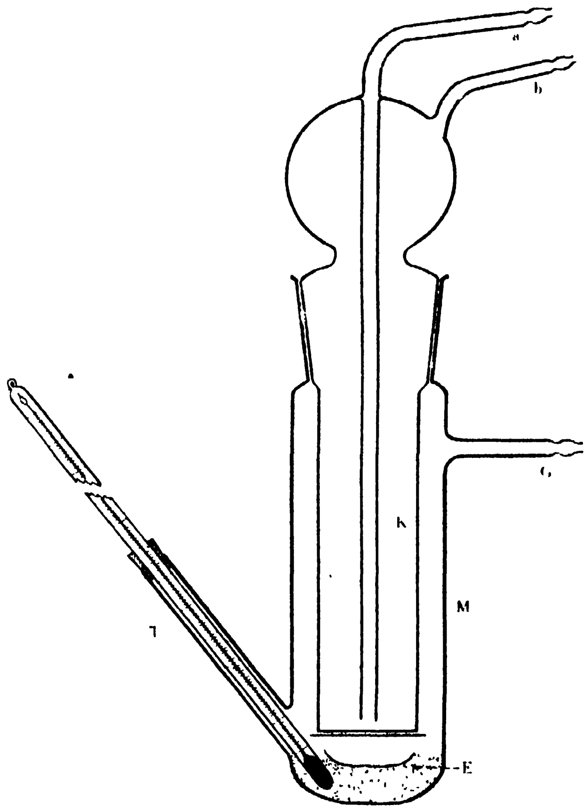

Fig. 1. Apparat zur Mikrosublimation unter vermindertem Druck.

Am Tubus (T) wird der Thermometer montiert, mit dem die Temperatur der Eisenfeilspäne (E) gemessen wird. Meiner Erfahrung nach beträgt der Unterschied der Temperatur zwischen Eisenfeilspänen und Paraffinölbad gewöhnlich 30–50°C, je nach der Stärke des Brenners. Nachdem man den Thermometer auf den Tubus gesetzt hat, zieht man unter vermindertem Druck die konzentrierte Kollodionlösung auf den Kork und den benachbarten Teil des Tubus über. Der Kautschukschlauch, der bei der Evakuierung verwendet wird, muss vorher einem künstlichen Alterungsprozess unterzogen werden, wozu man einen neuen Kautschukschlauch zuerst eine Zeit lang in verdünnte

Natronlauge legt und 1–2 Stunden lang aus einem Dampfentwickler lebhaft Wasserdampf hindurchstreichen lässt.

Zur Aufnahme des Versuchsmaterials dient ein flaches Schälchen (Durchmesser 28 mm), das aus 0.3 mm starkem Kupfer- oder Platinblech hergestellt wurde. Zum Auffangen des Sublimats wird ein rundes Deckglas (38 mm) verwendet, das mit einem Tropfen wasserfreien Glyzerins an der ebenen untersten Kühlfläche angeklebt wird. Der Abstand vom Boden des Schälchens bis zum Deckglas soll 4–5 mm betragen. Um das Festsitzen des Kupfer- oder Platinschälchens zu sichern und um die Wärmeleitung zu beschleunigen, befinden sich am Boden des Mantels feine Eisenfeilspäne. Da aber diese Eisenfeilspäne manchmal mit schmierigen Substanzen gemischt in den Handel kommen, so muss man sie vorher mit Äther mehrmals reinigen. Die vollständig getrockneten Eisenfeilspäne legt man auf den Boden des Mantels und steckt dahinein die Kugel des Thermometers.

Der Apparat wird zum bequemen Gebrauch auf einem Stativ befestigt. Das Anheizen geschieht in einem Paraffinölbad, wobei der untere Teil des Apparats 3 cm tief eingetaucht wird. Die seitliche Tubulierung des Mantels (G) führt mit einem Kautschukschlauch unter Zwischenbindung des sog. Trockenapparats mit Kalziumchlorid, desgleichen mit Natronkalk und des Manometers, zu der Cenco Hyvac. Pumpe.

B. Verlauf der Sublimation.

Etwas Versuchsmaterial wird in ein Kupfer- oder Platinschälchen gelegt, genau gewogen und, wenn Salze der Oxalsäure in Frage kommen, je nach dem Gewicht des Versuchsmaterials mit einigen Tropfen konzentrierter Phosphorsäure versetzt, mit der Nadel fein zerzupft und bei 60°C 15 Minuten lang getrocknet. Dabei muss man vorsichtig sein, nicht zu viel Phosphosäure hinzuzufügen. Das so behandelte Schälchen wird mit einer langen Pinzette auf die Eisenfeilspäne stellt. Zunächst wird das vorher gewogene Deckglas mit einem Tropfen wasserfreien Glyzerins an der ebenen untersten Kühlfläche angeklebt.

Der Kühler wird in einen Mantel gesteckt, Wasser zugeleitet und dann der Boden des Mantels 3 cm tief ins Paraffinölbad eingetaucht. Zum Evakuieren des Mantelraums wird ein Motor angetrieben, bis der Innendruck 5 mm beträgt, danach wird der Apparat bis auf 100°C erhitzt und erst dann der Brenner reguliert, um die Wärme 30 Minuten auf 110°C zu halten. Um den Luftstrom zu erzeugen, wurde während dieser Zeit der Motor alle 5 Minuten etwa eine Minute lang angekurbelt. Nach 30

Minuten langem Sublimieren unterbricht man die Erhitzung, nimmt den Apparat aus dem Paraffinölbad und lässt ihn auf Laboratoriumstemperatur abkühlen. Erst dann wird durch zwei sog. Trockenapparate langsam Luft eingeleitet (wenn man sie schnell einleitete, würden die Eisenfeilspäne hochfliegen und sich ans Deckglas ansetzen) und mit Vorsicht der Kühler aus dem Mantel herausgenommen. Das Deckglas wird vom Kühler abgenommen, die vom Sublimat beschlagene Seite nach oben gelegt und das Glyzerin zunächst mit nasser und dann mit trockner Gaze und zum Schluss nochmals mit Hirschleder gut abgewischt. Das so bearbeitete Deckglas mit Sublimat legt man in eine Schale von 45 mm Durchmesser, deren Boden mit schwarzem Papier bedeckt ist, und lässt sie im Wägezimmer eine Stunde lang stehen und wiegt dann genau.

Das vermehrte Gewicht ist als die gesuchte Gesamtmenge reiner Oxalsäure, die sich in freier oder gebundener Form im Versuchsmaterial vorfand, anzusehen. Dass dieses Sublimat von reiner Oxalsäure herrührt, kann man leicht durch eine $Sr(NO_3)_2$- oder $Ca(NO_3)_2$-Lösung nachweisen, weil das Sr- oder Ca-Salz der Oxalsäure sehr charakteristisch ist. Die Sublimate erhält man auf dem Deckglas als scharf umgrenzte, kreisförmige Beschläge von etwa 27 mm Durchmesser. Da das Deckglas an der Kühlläche von 33 mm Durchmesser angeklebt war, so ist es höchst wahrscheinlich, dass fast restlos kein seitliches Ausstreichen der Dämpfe erfolgen konnte.* Eine Zersetzung oder Verflüchtigung der einmal an der Vorlage niedergeschlagenen Sublimate durch Temperaturerhöhung fällt hier begreiflicherweise weg. Um die Säuren der gebundenen Form durch Sublimation rein darzustellen, fügt man zur Abspaltung der Salze konzentrierte Phosphorsäure hinzu, wodurch keine Zersetzung der Substanzen eintritt, sodass im Sublimat dasselbe Produkt wie bei der freien Säure zu finden ist. Die Empfindlichkeit bleibt dieselbe wie bei der freien Säure und wird von der Schwankung der Sublimationstemperatur nicht beeinflusst. Die Ausbeute aus den Oxalsäureverbindungen beträgt z. B. beim Oxalammon (neutr.) 99.02%, beim Kaliumoxalat (neutr.) 98.51% und beim Kalziumoxalat 98.09%.

Die Ausbeute aus reiner, mit Phosphorsäure versetzter Oxalsäure beträgt 98.85%. Der Kontrollversuch, bei dem durch Zusatz von 5 qmm Filtrierpapier auf reine Oxalate und Phosphorsäure ein den natürlichen Pflanzengeweben möglichst ähnlicher Zustand geschaffen wurde, gab ganz befriedigende Ausbeute: bei Oxalammon (neutr.) 98.62%, bei Kaliumoxalat (neutr.) 98.72% und bei Kalziumoxalat 98.09%.

Zusatz von Phosphorsäure hat hier neben der Aufspaltung der Oxalate

noch den Vorteil, dass dadurch das Gewebe in einen homologen Brei verwandelt wird, bei dem die Sublimation leicht vor sich geht. Durch diese Methode ist es leicht, die unlöslichen Oxalate von den löslichen zu trennen, indem man die löslichen Säureanteile durch 10–15 Minuten langes Kochen im destill. Wasser auszieht.

IV. VERSUCHSERGEBNISSE.

Versuch 1.

Die Versuchspflanzen wurden im Gewächshaus hinter aus Phragmites-Stengeln hergestellten Vorhängen kultiviert, um sie vor zu starkem Licht zu schützen. Gewöhnlich wurden dabei für jede Reihe, mit Ausnahme ganz weniger Fälle, drei oder vier Individuen genommen. Es wäre gerade für unseren Zweck wünschenswert gewesen, nach dem Kulturbeginn alle zwei oder drei Wochen die Schwankungen des Säuregehalts der Versuchspflanze zu verfolgen; leider geschah es aus ganz äusserlichen Gründen nicht, sodass es noch Aufgabe späterer Forschung bleibt. Tabelle 2 stellt das Ergebnis aus dem Individum nach 48 Tagen Züchtung (vergl. Fig. 2) dar. Die Zahlen geben die Gesamtmenge der Oxalsäure sowohl aus löslichen als aus unlöslichen Oxalaten. Zwischen den einzelnen, unter ganz gleichen Bedingungen lebenden Individuen war in bezug auf ihren Säuregehalt nur kleiner Unterschied wahrzunehmen.

Aus umstehend angegebenen Zahlen ersieht man, dass der Säuregehalt des Blattstiels immer grösser als der der Blattspreite ist, während er im Stengel bedeutend geringer als in den Blättern ist. Weiter kann man sehen, dass er in ganz jungen Blattspreiten und Blattstielen geringer als in mässig gewachsenen ist. In jedem Falle ist aber sowohl bei Blattspreite als auch bei Blattstiel die allgemeine Neigung zur Abnahme des Säuregehalts entsprechend der Reihenfolge der Knoten von der Basis nach der Spitze zu wahrzunehmen. Was den Säuregehalt des Stengels einschliesslich der betreffenden Knoten betrifft, so liegt es damit gerade umgekehrt, er nimmt von der Basis nach der Spitze hin zu. Diese Beziehung ist aus der letzten Spalte der Tabelle 2 oder aus Fig. 3 ersichtlich, wo der Säuregehalt im Prozentsatz zum Frischgewicht angegeben ist.

Ein ganz ähnliches Verhältnis findet sich bei den weiter entwickelten und schon zur Blüte gekommenen Individuen, wie Fig. 4 den prozentualen Säuregehalt der 98 Tage lang kultivierten Pflanzen wiedergibt. Der Säuregehalt der vollständig entwickelten Blüte ist geringer als der der halb entwickelten. Trotzdem ist er in der Blütenknospe sogar geringer

TABELLE 2.

Der Säuregehalt verschiedener Pflanzenteile von *Begonia Evansiana* ANDR. nach 48 tägiger Züchtung im Gewächshaus.

Nummer des Schälchens	Pflanzenteile	Frischgewicht des Materials (g)	Gefundene Oxalsäure (g)	Prozent
1	1ste Blattspreite	0.123615	0.000610	0.4932
2	,,	0.194570	0.000927	0.4763
3	,,	0.159790	0.000851	0.5325
4	,,	0.245715	0.001292	0.5258
5	,,	0.272435	0.001402	0.5146
38	2te Blattspreite	0.110493	0.000525	0.4751
25	,,	0.150767	0.000749	0.4972
26	,,	0.163492	0.000835	0.5107
27	,,	0.120530	0.000599	0.4973
28	,,	0.188270	0.000764	0.4288
41	,,	0.217355	0.000905	0.4163
43	,,	0.196460	0.000782	0.3985
46	3te Blattspreite	0.152180	0.000460	0.3027
47	,,	0.139132	0.000[illegible]39	0.3655
48	,,	0.145902	0.000488	0.3344
49	,,	0.159595	0.000547	0.3427
50	,,	0.225615	0.000675	0.2991
51*	,,	0.246920	0.000763	0.3090
52	,,	0.279565	0.000815	0.2915
32	4te Blattspreite	0.080910	0.000294	0.3633
33	,,	0.176564	0.000691	0.3913
20	5te Blattspreite	0.107082	0.000175	0.1632
21	,,	0.140310	0.000358	0.1489
6	1ster Blattstiel	0.093745	0.000512	0.5462
16	,,	0.108370	0.000598	0.5522
44	2ter Blattstiel	0.127605	0.000652	0.5109
45	,,	0.124100	0.000626	0.5044
53	3ter Blattstiel	0.219483	0.000888	0.4045
34	4ter Blattstiel	0.057650	0.000223	0.3863
23	5ter Blattstiel	0.068186	0.000168	0.2462
31	,,	0.052870	0.000133	0.2515
76	Stengel einschliesslich 1ster Knote	0.205330	0.000367	0.1783
75	2ter Knote	0.265310	0.000574	0.2162
74	3ter Knote	0.161267	0.000488	0.3023
54	4ter Knote	0.173015	0.000606	0.3502
37	5ter Knote	0.132432	0.000588	0.4440

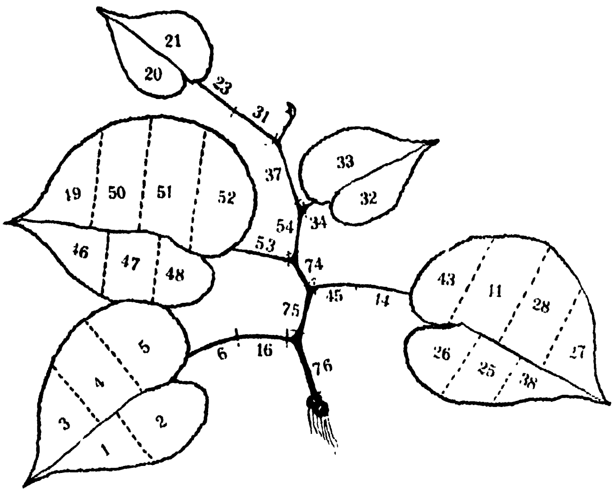

Fig. 2. Die Pflanze nach 48 Tagen Züchtung. Die Nummer bezieht sich auf das Schälchen, in welchem der betreffende Pflanzenteil aufgenommen wurde. $\times$ 1/3 .N. G.

als in der vollständig entwickelten Blüte. Im Gegensatz zum Blattstiel und zur Blattspreite verhält sich der Säuregehalt im Blütenstiel zu dem der eigentlichen Blüte gerade umgekehrt, indem jener eben geringer als dieser war.

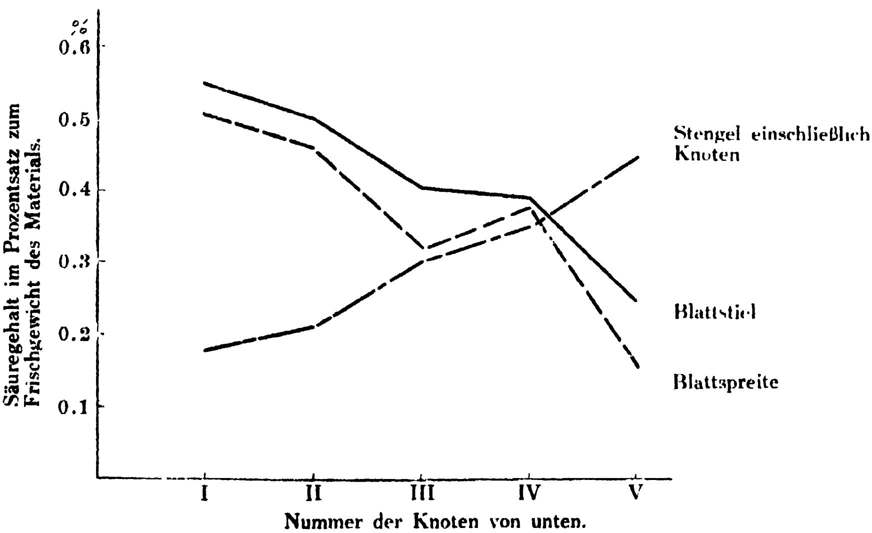

Fig. 3. Der Säuregehalt verschiedener Pflanzenteile von *Begonia Evansiana* Andr. (vergl. Tabelle 2.)

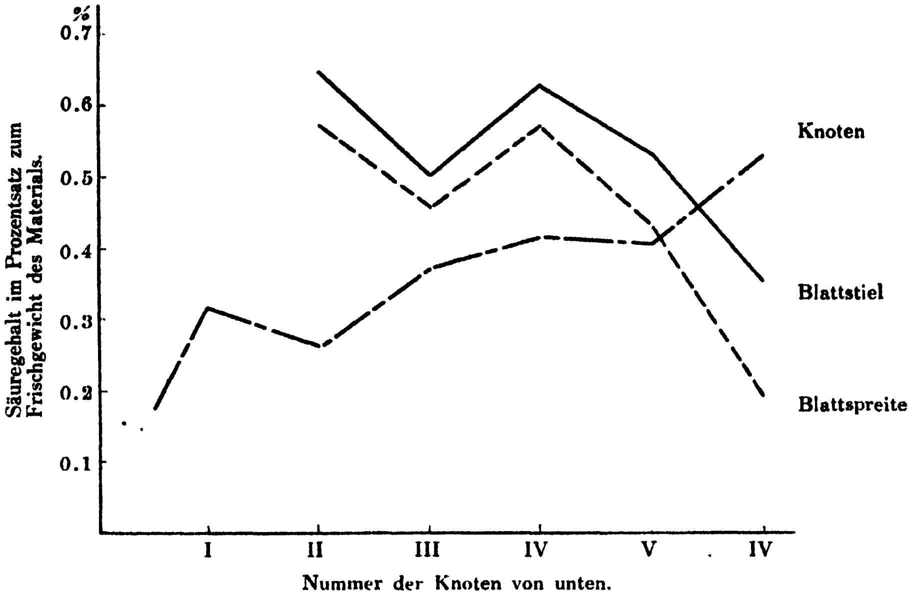

Fig. 4. Der Säuregehalt verschiedener Pflanzenteile von *Begonia Evansiana* ANDR. nach 98 tägiger Züchtung im Gewächshaus.[1]

Es ist aber zu betonen, dass der Säuregehalt der Blüten im allgemeinen viel grösser als der aller anderen Teile des Pflanzenkörpers war, soweit meine Versuche reichten. Bei diesem Individuum ist, statt des Säuregehalts des Internodiums einschliesslich der Knoten, der der Knoten allein bestimmt, weil die Säure sich in den Knoten viel reicher als im Internodium findet. Der Säuregehalt nimmt in den Knoten von der Basis nach der Spitze hin zu. In untersten Stengelteil, der dem Bulbillum am nächsten steht, findet sich ganz wenig Säure.

Versuch 2.

Die jungen Pflanzen wurden aus dem Eisschrank genommen, gleich in ein Kulturgefäss gesetzt und im Keller unter Lichtabschluss weiter kultiviert. Dabei wuchs die Pflanze mit überlangem, elfenbeinweissem Stengel, nur die Knoten waren rot, und die gelben Blätter waren sogar nach dem

[1] Es fehlt für den 1sten Knoten die Angabe des Säuregehalts sowohl im Blattstiel als auch in der Blattspreite gerade darum, weil das Blatt vorzeitig zu Grunde gegangen war.

Ablauf von 40 bzw. 44 Tagen noch nicht entfaltet. In Fig. 5 sind die Ergebnisse der Versuche mit den Pflanzen Nr. 70 (40 Tage Züchtung), Nr. 64 (44 Tage Züchtung) und Nr. 60 (79 Tage Züchtung) wiedergegeben.

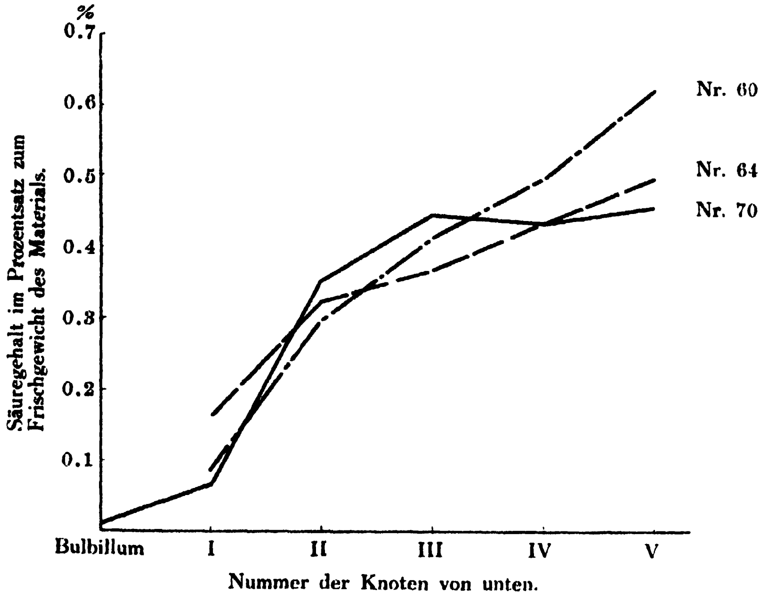

Fig. 5. Reihenfolge des Säuregehalts von *Begonia Evansiana* ANDR. im Dunkeln. Nr. 70 (40 Tage Züchtung) Nr. 64 (44 Tage Züchtung) Nr. 60 (79 Tage Züchtung)

Aus dieser Figur kann man vor allem sehen, dass der prozentuale Säuregehalt im Stengel einschliesslich des Knotens, des Blattstiels und der Blattspreite von der Basis nach der Spitze hin zunimmt. Weiter ist es offenbar, dass sich diese Zunahme besonders in der Spitzenzone mit der Dauer der Züchtung vergrössert, sodass der Säuregehalt bei der Pflanze Nr. 60 am grössten ist und danach bei Nr. 64 und dann bei Nr. 70.

Im Gegensatz dazu verhält sich der Säuregehalt am 2. Knoten gerade umgekehrt. Diese Tatsache spricht wahrscheinlich dafür, dass die aus den Reservestoffen gebildete freie oder gebundene Säure, oder daran kausal gebundene Vorgänge gerade nach den stärker wachsenden Körperteilen hin verschoben werden. Die Folge davon ist, dass die Kurve des Säuregehalts mit der Kulturdauer steiler, aber glatter verläuft.

Schliesslich ist aus Fig. 5 ersichtlich, dass die Oxalsäure sogar in Bulbillum (der Pflanze Nr. 70) nachweisbar war. Diese auffalende Er-

scheinung wird uns nur dadurch begreiflich gemacht, dass sie mit den Stoffwechselvorgänge bei der Entwicklung im Zusammenhang steht.

Versuch 3.

Um zu erkennen, ob und wie die Assimilation der Kohlensäure die Säureanhäufung beeinflusst, wurden die Versuche mit nachstehender Vorrichtung ausgeführt. Die Luft wurde zuerst durch ein 55 cm langes Glasrohr von 2.5 cm weitem Durchmesser, das mit Natronkalk gefüllt war, und durch zwei Flaschen, die mit je 1 l konzentrierter Kalilauge gefüllt waren, und weiter durch eine mit Barytwasser gefüllte Flasche durchgeleitet, um dadurch CO_2 freie Luft mit beinahe konstanter Stromgeschwindigkeit durch eine Glasglocke von ca. 20 l Inhalt, in der sich die Versuchspflanzen befanden, treiben zu können. Zur Kontrolle wurde gewöhnliche Luft durch ein mit Baumwolle gefülltes Glasrohr, durch drei Flaschen mit Wasser und eine Glasglocke, in der sich die Kontrollpflanzen befanden, durchgeleitet. Während der ganzen Versuchsdauer wurden die Kalilaugelösung, der Natronkalk und das Barytwasser mehrmals erneuert.

Unter diesen künstlichen Bedingungen wuchsen die Versuchspflanzen, wegen der mit Wasserdampf gesättigten Luft in der Glasglocke, etwas mangelhaft, und die Blattspreite war saftreich, dünn und blassgrün im Vergleich zu der frei im Gewächshaus gezogenen Pflanze. Nach dem Ablauf einer bestimmten Zeit wurde der Unterschied in ihren äusseren Merkmalen zwischen den Versuchs- und den Kontrollpflanzen ganz deutlich bemerkbar, und zwar in bezug auf die Grösse der Blattspreite, die Dicke und die Länge des Stengels und die Höhe aller Pflanzen usw.

Nachstehende Figur zeigt das analytische Ergebnis beim Individuum, das 183 Tage lang gezüchtet war. Wie man sieht, ist vor allem bei den Blättern und den Knoten der Säuregehalt der Kontrollpflanzen viel grösser als der in kohlensäurefreier Luft gezogener, und, gegenüber den geringen Schwankungen des Säuregehalts bei den in kohlensäurefreier Luft gezogenen Pflanzen in den einzelnen Zonen, nimmt er bei den Kontrollpflanzen von der unteren Zone nach der oberen hin stark zu. Diese Neigung war ganz unerwartet, denn bei den vorigen Versuchen war es gerade umgekehrt. In beiden Fällen ist aber der Säuregehalt des Blattstiels, dem vorigen Versuche ganz entsprechend, grösser als der der Blattspreite.

Nicht weniger unerwartet war auch, dass der Säuregehalt der Knoten gerade in umgekehrter Richtung von der Basis nach der Spitze hin abnimmt. Diese Erscheinungen können auf der Rückwanderung der Säure in die

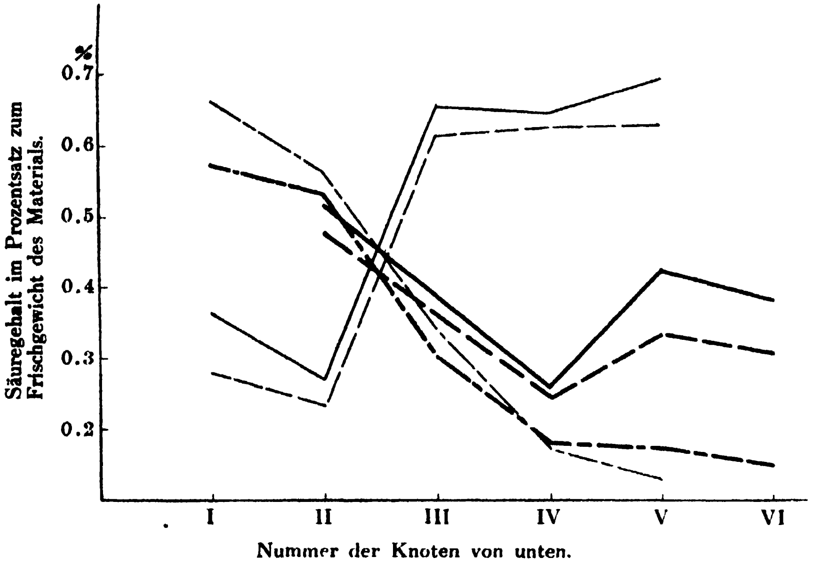

Fig. 6. Reihenfolge des Säuregehalts in CO_2 freier Luft gezogener Pflanzen und von Kontrollpflanzen (183 Tage Züchtung).

	In CO_2 freier Luft gezogene Pflanzen.	Kontrollpflanze.
Blattstiel.		
Blattspreite.		
Knoten.		

Knollen oder auf entgegengesetzten Stoffwechselvorgängen in diesen Pflanzen beruhen, sodass sie im allgemeinen gerade da hätten vorkommen sollen, wo nun das Wachstum zum Stillstand kam. Somit ist der Versuch noch nicht endgültig beendet, und die Frage bleibt noch offen, ob man aus dem gefundenen Unterschied des Säuregehalts bei beiden Fällen auf etwaige Beziehungen zwischen der Kohlensäureassimilation und der Säurebildung schliessen kann.

V. ZUSAMMENFASSUNG.

1. Der Mikrosublimationsapparat, den schon Klein und Werner für die Trennung und den qualitativen Nachweis organischer Säuren benutzt hatten, ist etwas verbessert und die Konstruktion des Apparats nebst dem Vorgang der Sublimation näher beschrieben worden.

2. Es gelang mir mit dieser Methode, die organischen Säuren (hier ist nur Oxalsäure berücksichtigt) in den pflanzlichen Geweben quantitativ

zu bestimmen.

3. Bei der reinen freien wie auch bei der reinen gebundenen Form der Oxalsäure konnten wir immer eine ganz befriedigende Ausbeute von ca. 99% wieder gewinnen.

4. Sowohl bei Bulbillen als auch bei Knollen von ***Begonia Evansiana*** ANDR. konnte keine Spur von Oxalsäure nachgewiesen werden.

5. In den übrigen Pflanzenteilen, wie Blättern, Stengel, Blüten und Kelchblättern usw., fand sie sich dagegen mehr oder weniger reichlich.

6. Im allgemeinen war der Säuregehalt in den Blüten am grössten. In bezug auf das Mengenverhältnis dieser Säure in den verschiedenen Entwicklungsstadien besteht folgende Reihenfolge:

Halb entwickelte Blüte>vollständig entwickelte Blüte>Blütenknospe.

7. Der Säuregehalt des Blattstiels ist immer grösser als der der Blattspreite. Er nimmt aber in beiden Fällen von den unteren Knoten her nach der oberen hin allmählich ab.

8. Der Säuregehalt im Stengel bzw. im Knoten nimmt bei wachsenden Pflanzen von der Basis nach der Spitze hin zu. Im untersten dem Bulbillum nächsten Stengelteil ist er nur ganz gering.

9. Bei den im Dunkel gezogenen Begonien nimmt der prozentuale Säuregehalt im Stengel einschliesslich des Knotens, Blattstiels und der Blattspreite von der Basis nach der Spitze hin zu, und diese Zunahme, besonders in der Spitzenzone, wird mit der Züchtungsdauer grösser.

10. Bei den in kohlensäurefreier Luft gezogenen Pflanzen nimmt der Säuregehalt der Blattspreite und des Blattstiels vom unteren Knoten nach dem oberen hin ab; bei den Kontrollpflanzen dagegen verhält es sich mit dem Säuregehalt gerade umgekehrt; er nimmt von der Basis nach der Spitze hin stark zu.

11. Sowohl bei der in kohlensäurefreier Luft gezogenen als auch bei der Kontrollpflanze verläuft die Reihenfolge des Säuregehalts in den Knoten ebenfalls in gerade umgekehrter Richtung zu dem der vorigen Fälle, und zwar nimmt er von der Basis nach der Spitze hin ab.

12. Diese unerwartete Erscheinung scheint dafür zu sprechen, dass der Säuregehalt je nach den verschiedenen Entwicklungsstadien ganz anders ausfällt, und dass beim Wachstumsaufhören infolge der umgekehrten Stoffwechselvorgänge sogar die umgekehrte Reihenfolge des Säuregehalts bestehen kann.

Vorliegende Untersuchung entstand auf Veranlassung und unter Leitung meines verehrten Lehrers, Herrn Prof. Dr. Y. YAMAGUTI. Sie wurde im Biologischen Institut der Kaiserlichen Tôhoku-Universität zu Sendai vom

Frühjahr bis zum Herbst 1931 ausgeführt. Zum Schluss möchte ich Herrn Prof. Dr. Y. YAMAGUTI für seine Anregung und seine vielseitigen Ratschläge meinen herzlichen Dank aussprechen.

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On the Blood Vascular System of the Earthworm in Japan, *Pheretima communissima* GOTO et HATAI.

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(With 13 text-figs.)

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INTRODUCTION.

The many detailed investigations on the blood-vascular system of the earthworm were made mostly on *Lumbricidae*, but for investigations on *Pheretima*, one of the most common forms in the orient, references are scarcely to be found except for the works of BAHL on *Pheretima posthuma* L. VAILLANT (1921) and of HERTLING on *Pheretima heterochaeta* MICH. (1921).

However, the arrangement of the blood-vascular system of *Pheretima communissima* GOTO et HATAI appears somewhat different from both species mentioned above and therefore I undertook this study to further extend my previous work on the physiology of this system (1930).

MATERIAL AND METHODS.

The material employed in the present work was *Pheretima communissima* GOTO et HATAI which is one of the most common earthworms in Japan.

In studying this system both dissection and serial paraffin sectioning were employed. As fixatives, sublimate alcohol, alcohol formol and trichlor acetic acid mixture after HEIDENHAIN were used. The latter gave the best results for my purpose.

RESULTS.

I. Observations on the First Thirteen Segments.

In this portion, the arrangement of the blood vascular system is highly modified from the rest of the body owing to the presence of complex and highly distributed sexual organs together with the strickingly differentiated alimentary canal. For the sake of convenience, I will describe the blood

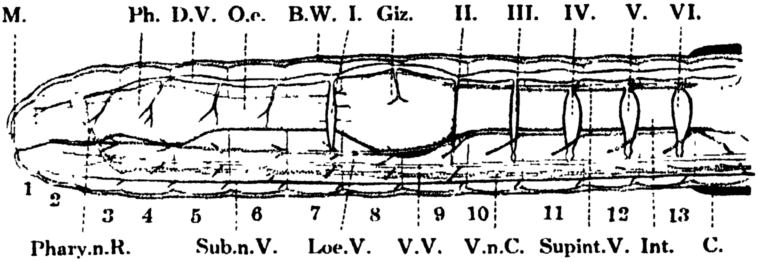

Fig. 1. Diagrammatic representation of the blood vascular system of the first thirteen segments.
M.=mouth, Ph.=pharynx, D.V.=dorsal vessel, Oe.=oesophagus, B.W.=body wall, Giz.=gizzard, I.-VI.=hearts, Phry.n.R.=pharyngeal nerve ring, Sub.n.V.=subneural vessel, Loe.V.=lateral oesophageal vessel, V.V=ventral vessel, V.n.C.=ventral nerve cord, Supint.V.=supraintestinal vessel, Int.=intestine, C.=clitellum.

vascular system in the first thirteen segments based on the diagrammatic figure (Fig. 1) which was reconstructed from the serial sections.

A. The longitudinal trunks.

In this category, the five main vessels may be included; that is, the dorsal, the ventral, the supra-intestinal, the lateral oesophageal, and the subneural vessels.

a. The dorsal blood vessel. This vessel runs along the dorsal median line of the body above the alimentary canal, and is distinctly separated from it in the first nine segments, but it lightly attaches to the dorsal surface of the intestine with a mesentery between Segment X and XIII. The wall of the vessel is thick and muscular, and is able to conduct peristalsis regularly. The foremost part of this vessel passes inside the pharyngeal nerve ring and bifurcates in the anterior Segment III. The bifurcated branches are remified into the portion of capillaries and distributed over the wall of the buccal cavity.

A pair of branches is given off just in front of each septum. The mode of branching in each segment is as follows:

In Segments III, IV, V, and VI: The dorsal vessel gives off a pair of small branches in these segments close to the anterior surface of each septum (Fig. 2, B).

In Segments VII, IX, and X: A pair of branches is given off from the dorsal vessel which is fairly larger than those branches given off in Segments III, IV, V, and VI. However, a pair in Segment IX is asymmetrical, and occasionally I found cases in which the one on the left side

is absent, while that in Segment X is always paired. These branches will be described more in detail in connection with the heart.

In Segment VIII: A pair of symmetrical branches supplies ramified capillaries to the wall of the gizzard, which are seen running longitudinally parallel to each other.

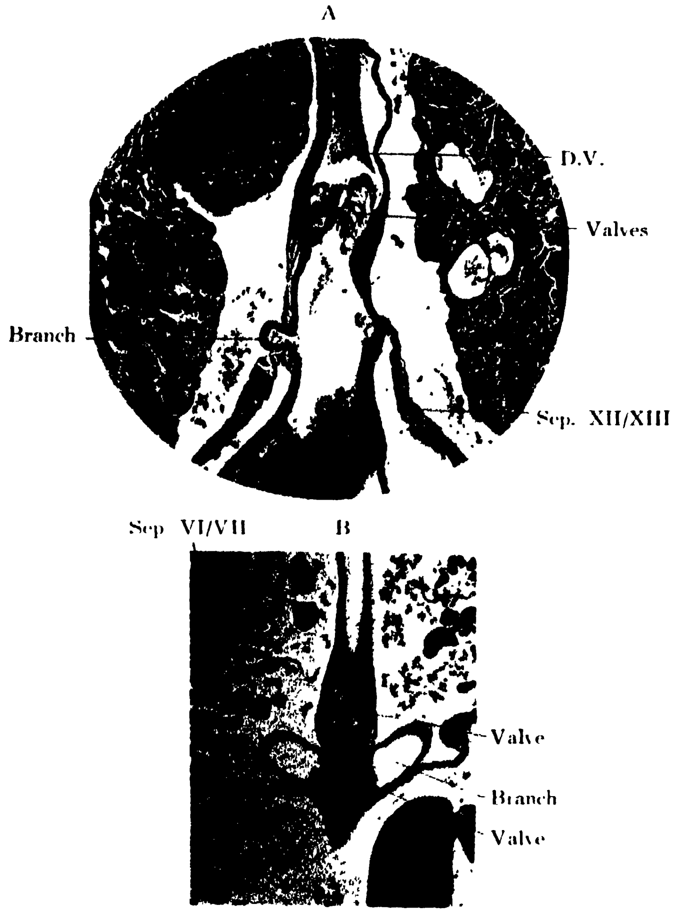

Fig. 2. A. Mode of branching and the valves of D.V. in Segment XII. B. In Segment VI. (Both frontal section).

In Segments XI, XII, and XIII: From the dorsal vessel branches off a pair of small vessels symmeterically in each segment. The branches are very small but their walls are considerably thick and muscular (Fig. 2, A) and are continuous with the hearts in these segments (Fig. 3 and 4).

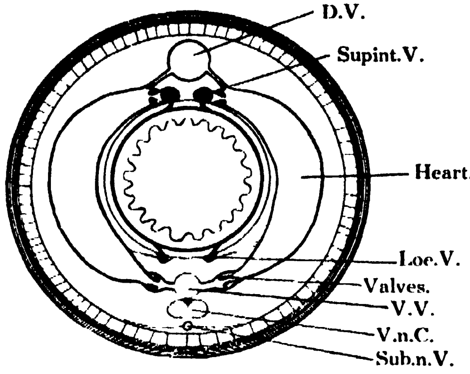

Fig. 3. Diagrammatic figure of the cross section of Segment XII.

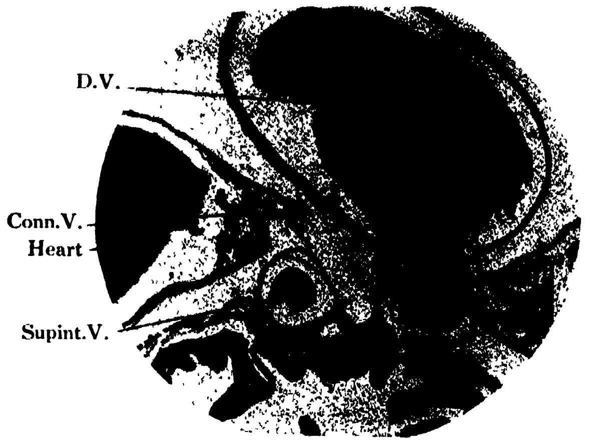

Fig. 4. The connection of the dorsal vessel and the heart in Segment XII. (Cross section).
Conn.V.=connecting vessel, hearts with the dorsal vessel.

On the valves in the dorsal vessel and its branches: A pair of well developed muscular valves which are directed forwardly, is situated regularly just in front of the branching point of the dorsal vessel in each segment (Fig. 2, A, B). The valves lie not only in the dorsal vessel, but are also found in the branches near their origin from the dorsal vessel and are directed centrifugally.

b. The ventral blood vessel. The ventral vessel is situated between the alimentary canal and the ventral nerve cord, in a position opposite the dorsal vessel. In Segment II, this vessel bifurcates and distributes

over the wall of the buccal cavity and the body wall. From this vessel a pair of branches, the ventro-tegumentary vessels, is given off at almost the same corresponding position as the branches of the dorsal vessel, with exceptions in Segments X, XI, XII, and XIII, in which these branches are not to be found (Fig. 1). Besides theses branches, a pair of hearts communicates with this vessel just in front of the posterior septum located in Segments IX, X, XI, XII, and XIII. There are no valves found along the whole length of the vessel.

c. The supra-intestinal vessels. The supra-intestinal vessels are short and extend from Segment X to XIII. These are located beneath the dorsal vessel and are intimately attached to the dorsal surface of the intestine. Anteriorly the vessels break up into capillaries which in turn distribute over the wall of the gizzard, and posteriorly they become continuous with the hearts in Segment XIII. These vessels are double and fuse here and there, though there are wide individual variations as to the number and position of fusion.

The supra-intestinal vessels give off 8–12 branches, or the so-called ring-vessels, in each segment. These ring-vessels enter the muscular wall of the intestine and become continuous with the lateral oesophageal vessels which are situated immediately on the ventro-lateral aspect of the alimentary canal. It seems certain that these vessels branch and fuse at several places in the wall of the intestine, because the number of the connections with the supra-intestinal and the lateral-oesophageal vessel varies even in the same segment. In addition to the ring vessel, the hearts are connected with the supra-intestinal vessels in Segments XI, XII, and XIII. This vessel has no valves.

d. The lateral oesophageal vessels. These vessels are two parallel longitudinal trunks and are situated on the ventro-lateral aspect of the alimentary canal in the first thirteen segments. In the first nine segments, the vessels are distinctly separated from the alimentary canal and in Segment III each vessel bifurcates at the lateral side of the pharyngeal nerve ring, while those in Segments X, XI, XII, and XIII are intimately attached to the intestine. From Segment XIV on, the vessels again separate from the intestine gradually, and the larger vessel which is situated on the right side runs around the ventral vessel and the ventral nerve cord, uniting itself to the subneural vessel at the region just in front of the posterior septum in Segment XIV, while the smaller vessel situated on the left side ends in capillaries which distribute over the body wall. The left vessel does not seem to become continuous directly with the subneural

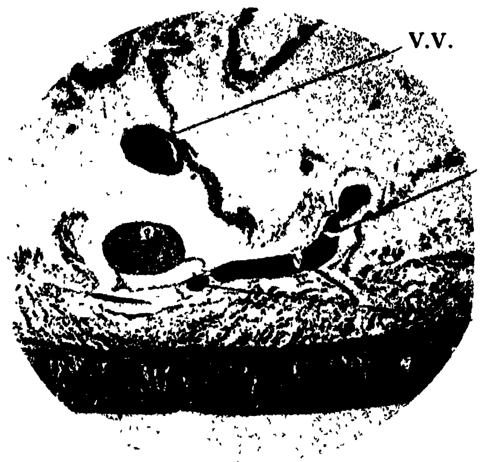

Fig. 5. This picture shows the connection of the lateral oesophageal vessel and the subneural vessel. (Cross section).

vessel as in the case of the right vessel (Fig. 5).

Similar as the other longitudinal vessels, the lateral oesophageal vessels give off many branches; one branch unpaired is issued outwardly in each of Segments IX, X, XI, XII, and XIII, and again a pair is found in each of Segments IV, V, VI, VII, and VIII, one of which runs inside, the other

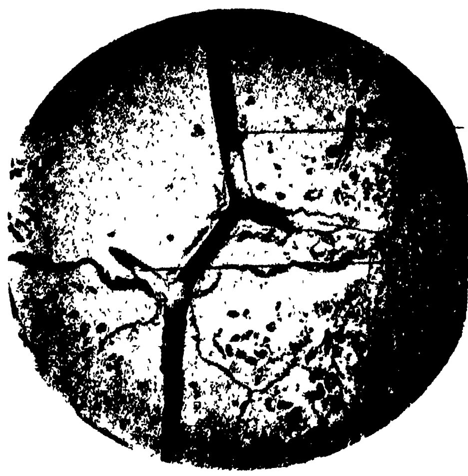

Fig. 6. The subneural vessel in Segments VII and VIII. (Frontal section).

being outwardly directed. These small vessels branch off at the region situated in front of the posterior septum in each segment. These vessels lack valves and furthermore these two lateral oesophageal vessels show no direct connections with each other at any place.

e. The subneural vessel. In this species, the subneural vessel which is located just beneath the ventral nerve cord, runs continuously in a fairly zigzag manner from the tail end to the head. It is considerably smaller in the first thirteen segments and ramifies into two branches in Segment II.

A pair of branches is given off asymmeterically before the posterior septum in each segment (Fig. 6). No valves are observed in this vessel and in its branches.

B. The hearts.

There are altogether six pairs of hearts in *Pheretima communissima*. Segments VII, IX, X, XI, XII, and XIII contain a pair, respectively. As seen from Fig. 1, not all pairs of hearts show connection with the same vessels, and, in addition, the valves within the hearts show also differences in different segments. I shall describe each heart separately.

In Segment VII: The heart in this segment connects the dorsal and the lateral oesophageal vessels in the posterior of this segment. A few small vessels are branched off from the hearts and distribute over the wall of the alimentary canal.

The muscular wall in the lower part is thin and poorly developed but in the upper part it is considerably well developed. A pair of valves is found in the middle region as well as in the origin from the dorsal vessel, but there are valves in the lower part as in the other hearts.

The mode of connection of the lower part of the hearts in this segment and the number of small vessels which are branched off, vary with the individual as will be described in detail in a subsequent paragraph.

In Segment IX: The hearts connect the dorsal with the ventral vessel directly below. Generally, these hearts are paired but the right one is larger and its muscular wall is better developed than the left one which is very slender and with a poorly developed muscular wall.

Two pairs of valves which are directed downwardly are situated, one pair in the most upper part and the other in the lower expanded region.

The variations which occur in this heart are presented in the following paragraph.

In Segment X: A pair of hearts join the dorsal with the ventral

vessel in front of the septum of Segment X/XI. Their connection with the supra-intestinal vessel is not distinct in this species.

The walls of the hearts are fairly well developed on both sides, but the vessels are slender like those in Segment VII. In general, the left vessel is more slender than that of the right side.

In Segments XI, XII, and XIII: A pair of hearts is found in these segments. The mode of connection differs considerably from all the others so far described owing to the fact that, dorsally the hearts in these segments are continuous both with the dorsal and the supra-intestinal vessels as shown in the figures 2 and 4.

Ventrally the heart becomes continuous with the ventral vessel in exactly the same relative position to the posterior septum as dorsally. The lowest part of the vessel shows an expansion of fair degree (Fig. 7).

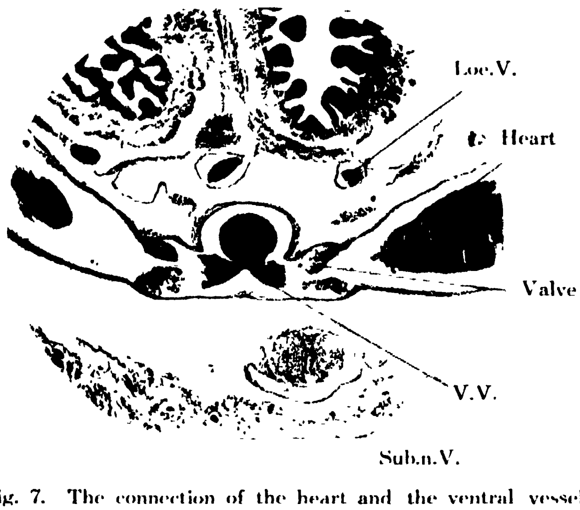

Fig. 7. The connection of the heart and the ventral vessel in Segment XIII. (Cross section).

The hearts are larger in caliber, possessing a thick and well developed muscular wall and in fact the contractile force is stronger than in the hearts in other segments.

A pair of valves directed downwardly is situated not only at the connecting region of the hearts with the supra-intestinal vessel as well as in the branches which are communicated with the dorsal vessel, but also in the lower expanded region in each segment (Fig. 3, 4 and 7).

II. Behind the Thirteenth Segment.

The blood vascular system behind the Segment XIII is relatively simple in comparison with that of the first thirteen segments, because the number of specialized organs is very few, and, in addition, these are arranged metamerically in nearly all segments. In this posterior region, there are found three longitudinal trunks, *i. e.* the dorsal, the ventral and the subneural vessels in the same relative positions as noted in the first thirteen segments. Fig. 8 shows the semidiagrammatic representation of general arrangement of the blood vascular system in the middle region of the body.

a. The dorsal vessel. This vessel as a continuation of the dorsal vessel found in the first thirteen segments also runs along the mid-dorsal line and attaches intimately to the alimentary canal with a mesentery from Segment XV. In the last segment the vessel first bifurcates and then subdivides into capillaries. The dorsal vessel receives a pair of commisural vessels from a dorso-lateral aspect in the most anterior part of each segment (Fig. 9). Besides these, two pairs of dorso-intestinal vessels join the dorsal vessel in a ventro-lateral aspect. The first pair entering one third of the distance from the anterior end of the segment and the other entering in a position two thirds distant (Fig. 10 and 11). But in the last segment of the body there is only one pair of dorso-intestinal vessels arising in the anterior one third portion. In Segment XIV, only one pair of dorso-intestinal vessels is found and the commisural vessel is aparently absent.

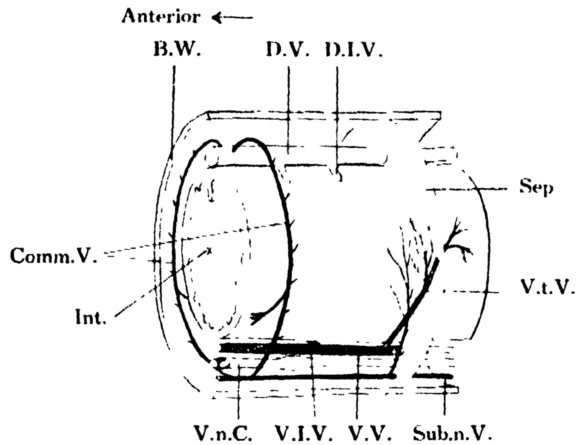

Fig. 8. Diagram representation of the blood vascular system in the middle region of the body.
D.I.V. = dorso-intestinal vessel, Comm.V. = commisural vessel, V.I.V. = ventro-intestinal vessel, V.T.V. = ventro-tegumentary vessel.

The wall of this vessel is well developed and the peristalsis traverses

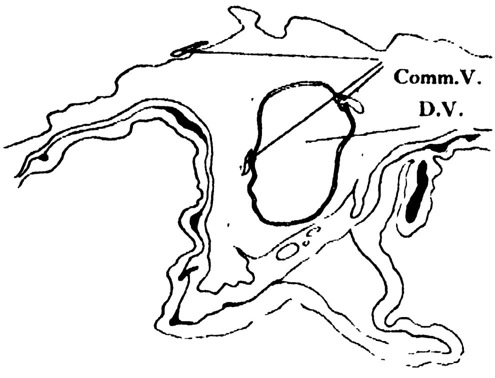

Fig. 9. Connection of the dorsal vessel and the commisural vessel. (Cross section).

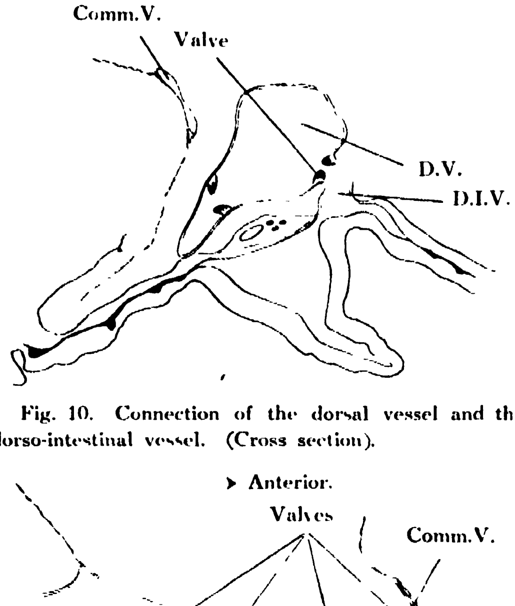

Fig. 10. Connection of the dorsal vessel and the dorso-intestinal vessel. (Cross section).

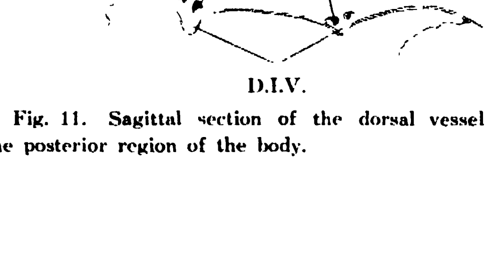

Fig. 11. Sagittal section of the dorsal vessel in the posterior region of the body.

from the posterior to the anterior.

A pair of intersegmental valves which are directed forwardly is situated in the posterior part of each segment with the exception of the last one. In addition, there is a pair of valves each at the entrance of the commisural and the dorso-intestinal vessels into the dorsal vessel.

b. The ventral vessel. The ventral vessel runs along the median ventral line and is attached to the intestine with a mesentery. Posteriorly it becomes slender and finally ends in capillaries.

This vessel gives off a pair of ventro-tegumentary vessels at the posterior part in each segment. The main vessel of the ventro-tegumentary vessel passes through the septum and enters the succeeding segment in which it branches off and sends many small vessels to the body wall. Another large branch which originates from the ventral vessel dorsally at the middle of the each segment is the ventro-intestinal vessel. It passes

diagonally upwards along the mesentery and becomes continuous with the intestinal blood plexus. In the last segment, there is no ventro-intestinal vessel.

The wall of the ventral vessel is thin and has no valves in it.

c. The subneural vessel. Among the longitudinal trunks, that of the subneural vessel is the most slender and runs along the mid-ventral line with a zigzag course beneath the ventral nerve cord as its name indicates. In the last segment it breaks up into capillaries. Two pairs of branches arise from this vessel: a larger pair connects the dorsal and the subneural vessels at the most anterior part of each segment and forms the commisural vessel while a smaller pair branches in the posterior one third portion of each segment and distributes well over the body wall, though in the last segment only a pair of commisural vessels is present.

d. The commisural vessels. The commisural vessels branch from the subneural vessel at the anterior end of each segment and pass upwards on the posterior face of the respective septum. As is shown in Fig. 12, it sends a branch medially which bifurcates and becomes continuous ultimately with the intestinal blood plexus. The main vessel continues to run almost entirely along the margin of the septum giving off some branches to the body wall, and finally enters the dorsal vessel in the dorso-lateral region as was described above.

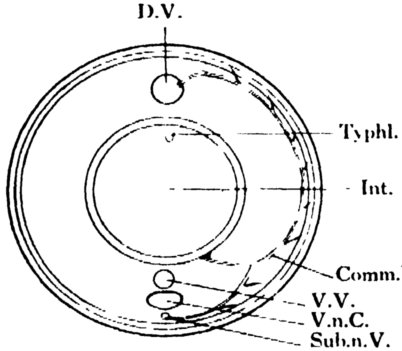

Fig. 12. Diagram figure of the commisural vessel.
Typhl. -typhlosoral vessel.

VARIATIONS.

Altogether several minor variations are noticed in relation to the arrangement of the blood vascular system. Those in the hearts of Segments VII and IX are most predominant.

Generally, there is a pair of symmetrical hearts in Segment VII, but occasionally in some individuals the left or the right heart is absent. The hearts of this segment continue with the lateral-oesophageal vessels in the manner as was described above, but in some cases they become

continuous with the ventro-tegumentary vessel directly instead of with the lateral oesophageal vessel (Fig. 13, B). Although the hearts exhibit a single tube, I found an individual with a portion of the heart, usually the ventral portion, though the exact location was variable, splitting and twisting on itself. This ventral portion of the heart was like a duplex twisted tube. In addition, one of these divided hearts became continuous with the lateral-oesophageal vessel and the other with the ventro-tegumentary vessel directly (Fig. 13, A). The valves at the junction of the heart and the dorsal vessel were absent occasionally. The number of the branches from the hearts varies from three to seven according to individuals, and even in the same individual the right or the left side shows variation.

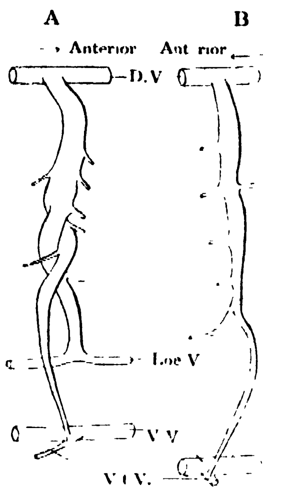

Fig. 13. Variation of the heart in VII segment

In the hearts of Segment IX, it was occasionally found that the smaller vessel in the left side was absent.

The dorsal vessel gives off a pair of branches in each segment, generally in the first thirteen segments, but sometimes two or three pairs of branches are given off in a segment. A similar variation often occurs in the lateral oesophageal vessel.

SUMMARY.

The blood-vascular system of *Pheretima communissima* GOTO et HATAI was investigated. This system may be divided into two regions, the first region includes the first thirteen segments (before clitellum) and the second region is behind the Segment XIII.

Ph. communissima has five longitudinal trunks, *i. e.* the dorsal, the ventral, the supra-intestinal, the lateral oesophageal, and the subneural vessel in the first region, but in the second region, the supra-intestinal and the lateral-oesophageal vessels are absent.

The longitudinal trunks give off or receive branches in each segment. The branches of these vessels are as follows:

The dorsal vessel in the first region gives off a pair of branches in each segment and receives a pair of commisural vessels and two pairs of

the dorso-intestinal vessels except in Segment XIV and the last segment in the second region. The ventral vessel gives off a pair of branches in each segment along the whole of its length, besides a single ventro-intestinal vessel in each segment of the second region. The subneural vessel gives off a pair in the first region and two pairs in the second region in each segment; the supra-intestinal vessel gives off many ring vessels in each segment, in addition, a pair of hearts in Segments XI, XII, and XIII. The lateral oesophageal vessels give off a single branch in Segments X, XI, XII, and XIII and a pair in the other segments.

There are five pairs of hearts connecting the dorsal and the ventral vessel, one each in Segments IX, X, XI, XII, and XIII, and, a pair connecting the dorsal and the lateral oesophageal vessel in Segment VII.

The dorsal vessel with its branches, and the hearts possess valves which turn towards the direction of blood flow.

I am greatly indebted to Prof. S. HATAI for his kind direction and advice given during the investigation.

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Report on a Collection of Sponges made in South Saghalin by Mr. TOMOE URITA.

BY

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(With Pls. VII–VIII and 6 text-figs.)

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The sponge fauna of the Asiatic waters to the north of Japan is practically unknown to us. A few records of sponges are known to us from the Behring Seas, and a few, of doubtful value, from the Sea of Okhotsk; and because Saghalin and the Sea of Okhotsk lie between the Arctic seas to the north and the warmer Japanese seas to the south, records from this area might be expected to yield interesting and important information. I am indebted therefore to Dr. SANJI HÔZAWA for the privilege of working through the collection of sponges made by Mr. TOMOE URITA in South Saghalin.

The specimens are deposited in the Biological Institute of the Tôhoku Imperial University.

The number of species represented in this collection is 8. Of these, 1 is believed to be cosmopolitan, or nearly so, 2 are new, 1 has been recorded hitherto only from Japan, 1 from Norway, and the remaining 3 are typically Arctic.

The list of species is: —

1. *Semisuberites arctica* CARTER.
2. *Uritaia*, gen. n. *halichondroides*, sp. n.
3. *Halichondria panicea* (PALLAS).
4. *Halichondriella corticata* BURTON.
5. *Eumastia sitiens* SCHMIDT.
6. *Suberites domuncula* (OLIVI).
7. *Rhizaxinella clavata* THIELE.
8. *Aplysinopsis lobosa*, sp. n.

Because the Saghalin area is likely to prove unusually interesting, when its fauna is more completely known, and in order to that there shall be no doubt as to the identification of the sponges herein recorded, I have included as many photographs as possible, even of the more familiar species.

1. Semisuberites arctica CARTER.

(Pl. VII, figs. 1–2; text-figs. 1–3).

Semisuberites arctica CARTER 1877, p. 39, pl. 1, fig. 1, a–c).
? *Veluspa polymorpha* var. *cribrosa* MIKLUCHO-MACLAY 1870, p. 6, pl. i, figs. 12, 13;
Cribrochalina variabilis et varr. *crassa, salpingoides* VOSMAER 1882, p 36, pl. i. figs. 16, 17, pl. iii, figs. 67–69, pl. iv, figs. 146–147; *C. sluiteri* LEVINSEN 1886, p. 14, pl. xxix, figs. 6–9, pl. xxx, fig. 6; *C sluiteri* SWARTZEWSKY 1906, p. 335, pl. xi, fig. 4, pl. xv, fig. 23; *C. variabilis* FRISTEDT 1887, p 418, pl. xxvi, fig. 4; *Stylaxia variabilis* TOPSENT 1913, p. 53; *Semisuberites arctica* TOPSENT 1919, p. 2.

Occurrence. — East Coast, off Sakaehama, South Saghalin, 20 fathoms; bottom, mud; September, 1929.

Remarks. — The species, although first described by CARTER, received a more complete description by VOSMAER (1. c.) when he established the species *Cribrochalina variabilis* for the reception of some sponges from the Barents Sea. These were divided two groups representing two varieties (varr. *crassa* et *salpingoides*) but it is evident, in view of the remarkable similarity between them, that this was quite unnecessary. In both varieties the sponges were stipitate, with cylindrical bodies ending in trumpet-shaped upper ends, the mouths of the trumpets being represented by shallow depressions bearing numerous oscula. A description of the skeleton was not given, but the spicules were stated to be styli which may occasionally become slightly subtylostylote. One of the co-types of the species is in the British Museum collection and from this the skeleton may be described

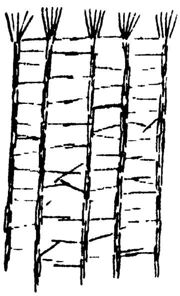


Fig. 1.

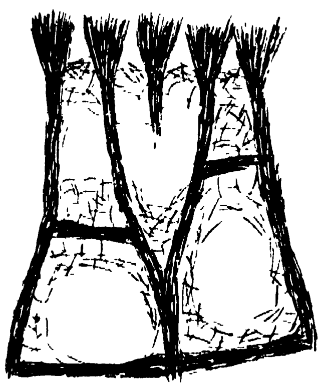


Fig. 2.

Text-figs. 1, 2. Sections at right angles to the surface to show the range of variation in the skeleton in *Semisuberites arctica* CARTER (Semi-diagrammatic). 1. From a co-type of *Cribrochalina variabilis* VOSMAER in the British Museum; 2. From the specimen collected by Mr. URITA at Saghalin.

as a subisodictyal reticulation of styli, with multispicular fibres running vertically to the surface and ending there in brushes of spicules. Isolated spicules scattered between the vertical fibres and arranged somewhat transversely to the primary fibres (text-fig. 1) give the effect of an isodictyum. The styli are variable in size and measure up to .36 by .008 mm.

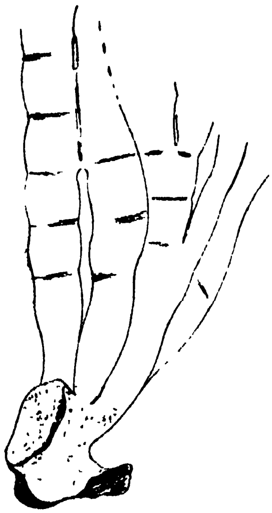
LEVINSEN recorded from the Kara Sea, under the name of *Cribrochalina sluiteri* VOSMAER, a number of specimens which obviously belong to *C. variabilis.* They differed from the type of the latter species in having a single oscule in the apical depression and in having spicules which vary from .2 to .64 mm. in length; and one of them was clavate rather than trumpet-shaped. It may be noted that LEVINSEN's suggestion that *C. variabilis* and *Auletta elegans* VOSMAER are synonymous with *C. sluiteri* is quite erroneous.

Other specimens have since been described by a number of authors, under various specific names. FRISTEDT's specimens varied from cylindrical, with cribriform oscular plate at the summit, to subinfundibuliform. SWARTZEWSKY's sponge was clavate like the one figured by LEVINSEN. TOPSENT described a number of specimens from Hope Island, near Spitzbergen, which appear to agree closely with those previously described, and in these the styli varied from .06 to .315 by .003 to .006 mm. One of these was remarkable for the long fibres, of spongin and spicules, found in the skeleton. In this work, TOPSENT created a new genus, *Stylaxia*, for the reception of *Cribrochalina variabilis.*

The specimen from Saghalin is stipitate and consists of a bunch of erect branches which vary from clavate to cylindrical or sub-infundibular. The height of the sponge is 14 cms. and the diameter of the branches varies from 1 to 2.5 cms. Here and there oscula are found on the sides of the branches but in all cases the summit of the branch bears a cribriform oscular plate (pl. VII, fig. 2). The branches show a tendency to fuse at the points where they meet.

The surface of the specimen is even, but the branches show an annular outline in places. The skeleton consists of a coarse reticulation of spiculofibre with a dense palisade of dermal brushes (text-fig. 2). The styli measure up to .3 by .007 mm. The presence of copious spongin in the skeleton shows that this specimen approximates closely to the fibrous specimen described by TOPSENT.

Veluspa polymorpha var. *cribrosa* MIKLUCHO-MACLAY (l. c.), so inadequately described, is probably synonymous with this species and has a close resemblance to the present specimens.



Text-fig. 3. The lower part of the Saghalin specimen of *Semisuberites arctica* CARTER, to show the dead portions (shaded) of an older sponge attached to the base of the present individual.

An interesting feature of the present specimen is that it has quite evidently grown from the base of another, presumably larger individual, that the latter had died and the remains of its base are left. The dead tissues extend for a short way up the stalks of the living branches, and these can be recognised by their lighter colour and greater compressibility. (text-fig. 3).

Distribution.—Barents Sea; Spitzbergen; White Sea; Okhotsk Sea (?).

Genus URITAIA, gen. n.

Genotype. — *U. halichondroides*, sp. n.

Diagnosis. — Axinellidae with skeleton composed of two categories of smooth styli; main skeleton a halichondroid reticulation of large styli, with a few small styli scattered between; dermal skeleton formed of brushes of smaller styli set at right angles to surface with numerous styli of similar size lying horizontally and scattered between them.

Remarks. — The genotype has a strong resemblance to *Amorphilla halichondroides* THIELE, and was at first sight mistaken for it. For this reason, a discussion of the systematic position of THIELE's species is given below under *Hymeniacidon.*

Genus HYMENIACIDON BOWERBANK.

Genotype. — *Spongia sanguinea* Grant.

Diagnosis. — Axinellidae with skelekton of smooth styli, not differentiated into categories, forming a halichondroid main skeleton and a special tangential, dermal skeleton.

Remarks. — In the majority of specimens of *Hymeniacidon sanguinea,* the dermal skeleton is easily seen, though in a few it is not strongly marked and is difficult to observe. The presence of this dermal skeleton appears to have been entirely ignored until THIELE (1898, p. 44) founded the genus *Amorphilla* for species which differed, according to him, from those of *Hymeniacidon* in the presence of a tangential dermal skeleton.

Under the circumstances therefore, *Amorphilla* must be regarded as a synonym of *Hymeniacidon*.

2. **Uritaia halichondroides**, sp. n.

(Pl. VII, figs. 3–4; text-fig. 4).

Holotype. — Spec. No. 9 (Pl. VII, fig. 4). In the collection of the Biological Institute, Tôhoku Imperial University.

Diagnosis. — Sponge irregularly massive; surface thrown into irregular ridges or tubercles; oscules few, small, inconspicuous, level with surface; colour, in spirit, ash-grey; main skeleton loose and irregular, dermal skeleton a close-set palisade of brushes of spicules; spicules slightly curved divided into 2 categories measuring .42 by .014 mm. and .26 by .007 mm. respectively.

Remarks. — In external appearance the holotype is practically identical with the specimen of *Halichondria panicea* shown in Pl. VII, figs. 5–9.

Text-fig. 4. *Uritaia halichondroides*, sp. n. spicules. ×200.

3. **Halichondria panicea** (PALLAS) Auctt.

(Pl. VII, figs. 5–9).

Occurrence. — East Coast, Sirutoru, depth unknown. Aniwa Bay, Tobuti, Shallow watter; bottom, rock: Aug., 1928. Off Sakaehama, 20 fathoms; bottom, mud; 1930.

Remarks. — There are three specimens, quite typical in anatomical details. The first is in every way like the specimen figured by BOWERBANK (1874, pl. xxxix, fig. 4), the second is intermediate in character between that figured on pl. xxxix, fig. 5 and pl. xi, fig. 5, and the third has essentially the characters of *Hymeniacidon firmus* BOWERBANK (l. c., pl. lxxii, fig. 1), which may be considered a synonym of *Halichondria panicea.*

There is a doubt about the actual distribution of this species, owing to the possibility of the wrong identification of specimens bearing a superficial resemblance to it. Under the circumstances therefore, it has been considered worth while to publish photographs of the present specimens in order to emphasize the truly striking resemblance to European examples of the species.

Photographs are included also of specimens from Misaki, Japan, collected from the littoral zone by Mr. INSOLE in May 1921 and deposited in the British Museum. These show beyond doubt that the species extends also to Japan.

There can be little doubt that *Spuma borealis* var. *tuberosa* MIKLUCHO-MACLAY (1870, p. 14, pl. ii, figs. 27–29) is synonymous with this species; and *S. borealis* var. *velamentosa* MIKLUCHO-MACLAY (l. c., p. 14, pl. ii, fig. 30) may be also.

Distribution. — Almost cosmopolitan (?).

4. Halichondriella corticata BURTON.

(Pl. VII, fig. 10).

H. corticata BURTON 1931 (M. S.)

Occurrence. - Aniwa Bay, off Merei, 10 fathoms, gravel, August 1928.

Remarks. — The specimen is an elongated, tuberose sponge, growing around a piece of seaweed. The surface is much wrinkled and bears several small, inconspicuous oscules scattered over the surface. The skeleton differs little from the holotype except that the dermal skeleton is denser and multispicular and the subdermal palisade of spicules is absent. These differences have probably little significance and may be only the result of different growth stages.

The holotype was fragmentary so that it is useful to have a record of a complete specimen.

Distribution. — Norway.

5. Eumastia sitiens SCHMIDT.

(Pl. VIII, figs. 11-12).

? *Spuma borealis* var. *papillosa* MIKLUCHO-MACLAY 1870, p. 13, pl. ii figs. 23, 24; *Eumastia sitiens* SCHMIDT 1870, p. 42, pl. v, fig. 12; FRISTEDT 1887, p. 426, pl. xxiv, fig. 13, pl. xxvii, fig. 13; LAMBE 1894, p. 115; Id. 1896, p. 182, pl. i, fig. 1; LUNDBECK 1902, p. 31, pl. iv, figs 1-6, pl. x, figs. 9–12; ARNESEN 1903, p. 6, pl. i, fig. 1, pl. vii, fig. 1; SWARTZEWSKY 1906, p. 333, pl. xv, fig. 21; HENTSCHEL 1929, p. 994; BURTON 1930, p. 496.

Occurrence. — East Coast, off Sakaehama, 20 fathoms; mud; September, 1929.

Remarks. — There are two specimens, both massive and bearing a number of papillae. The spiculation in each is typical but in external appearance they differ slightly. The first is irregular in form and measures 9 cms. long by 6 cms. across by 5 cms. high. The texture is soft and

fragile, the dermal membrane readily ruptured and torn from the underlying tissues and the papillae small and not particularly conspicuous. It agrees closely with the specimen figured by ARNESEN (l. c., pl. VII, fig. 1).

The second specimen approximates more closely to the one figured by LUNDBECK (l. c.). It is subspherical, about 4 cms. in longest diameter, firm and incompressible, not easily broken, and bears a number of conspicuous papillae. In no case is it possible to detect the characteristic oscula on the papillae, but this may be the effect of shrinkage due to preservation.

The two specimens are of particular interest in that they suggest an explanation of the true systematic position of *Spuma borealis* var. *papillosa* MIKLUCHO-MACLAY (1870, p. 13, pl. II, figs. 23, 24). The curious drawings meant to represent this variety might very easily have been made by an unskilled artist from the two specimens here described and there is every probability that *Spuma borealis* var. *papillosa* is synonymous with *Eumastia sitiens*.

Distribution. — Norway, Greenland, N. E. Coast of Canada;

6. Suberites domuncula (OLIVI).

(Pl. VIII, fig. 13).

(For further synonymy see TOPSENT 1900, p. 225)

? *Ficulina ficus* (Linnaeus) *sensu* TOPSENT 1900, p. 203.

Occurrence — Aniwa Bay, off Merei, 10 fathoms; gravel; December, 1928.

Remarks. — The single specimen is a large irregular mass bearing a number of lobose outgrowths and having at one point a wide opening leading into an internal chamber. The internal chamber lodges a hermit crab and the opening into it is sub-oval and measures 3 cms. by 1.5 cms. There is a second opening, on the opposite side of the sponge, measuring 7 mm. in diameter which is presumably the oscule. The specimen itself, which is a drab-grey in colour, measures 8 cms. by 7 cms. by 6 cms. The skeleton is indistinguishable from that of the European individuals of *Suberites domuncula* (OLIVI). There would be therefore no hesitation in assigning this specimen to OLIVI's species but for one circumstance, that THIELE (1898, p. 38) has described from Japan a sponge which resembles the present specimen in all respects but has in addition microstrongyla for microscleres. This sponge THIELE has referred to *Suberites suberea* (JOHNSTON), a species synonymous, according to TOPSENT (1900, p. 204), with *Ficulina ficus* (LINNAEUS). We have therefore the truly anomalous

state of affairs in which two sponges practically identical in all respects are referred not only to separate species but to different genera. The question naturally arises as to whether the presence or absence of a microstrongyla is sufficiently important to form the basis of a generic distinction. Having examined a number of specimens of *Ficulina ficus*, it is possible to say positively that these microscleres, as might have been suspected, vary considerably in the numbers in which they may be present. In one specimen they may be abundant, in another only a few will be present, and in a third they may be so rare that only careful and prolonged search will reveal them. For taxonomic purposes therefore they are valueless and *Ficulina* must be regarded as a synonym of *Suberites*.

There is yet another important point to be discussed. TOPSENT (l. c.) has included *Halichondria suberea* JOHNSTEN (1842, p. 139) as a synonym of *Ficulina ficus* (LINNAEUS), yet the sponges figured by JOHNSTON in this instance are to all intents identical with those figured by TOPSENT (l. c.) for *Suberites domuncula* (OLIVI). If now we compare TOPSENT's description of the latter with his description of *Ficulina ficus*, it becomes apparent that there is little to choose between the two species. They have the same texture, the same spiculation except for the presence of microstrongyla in *F. ficus*, and the gemmules in each case appear to differ little in structure or in the position they occupy within the maternal tissues. The only differences between them, apart from the question of the microstrongyla, are that *Ficulina ficus* is not invariably associated with mollusc shells, although it is in the majority of cases, and its shape is more irregular. *Suberites domuncula*, on the other hand, grows invariably around a mollusc shell in which a hermit crab usually dwells, and the shape is more consistently regularly massive and rounded, with no lobos outgrowths. Taking also into consideration the evidence afforded by a comparison between the specimen described here from Saghalin, and THIELE's specimens from Japan, it seems most probable that *Ficulina ficus* and *Suberites domuncula* are merely forms of a single species. At all events, the two species are congeneric and very closely related.

Distribution. — Europe; W. Indies; Senegal; Behring Straits; Japan, N. Pacific; Australia (fide TOPSENT 1900, pp. 208, 226).

7. Rhizaxinella clavata THIELE.

(Pl. VIII, fig. 14).

R. clavata THIELE 1898, p. 34, pl. i, fig. 19, pl. v, fig. 27, pl. viii, fig 1; *R. excellens* Id. l. c., p. 34, pl. iii, fig. 2, pl. viii, fig. 2; *R. arborescens* Id. l. c.,

p. 35, pl. iii, fig 3 b, pl. viii, fig. 3; *R. elevata* Id 1. c., p. 35, pl. iii, fig 3 a, pl. viii, fig. 4; *R. incrassata* Id. 1. c., p. 36, pl. iv, fig. 6, pl. viii, fig. 5; *R. cervicornis* Id. 1. c., p. 36, pl iii, fig. 4, pl. viii, fig. 6.

Occurrence. — East Coast, Sirutoru, depth unknown, 1930.

Remarks. — The six species described by THIELE (1. c.) are, in my opinion, identical. Their spiculation, as also the general appearance of the surface, are alike. All are erect and branching with a tendency to swell out slightly at the tips of the branches. The differences between them all are therefore slight. In *R. cervicornis* we have a sponge with thick branches, slightly flattened in places, and in *R. elevata* the branches are slender and slightly swollen at the tips, but these two sponges have everything else in common. Moreover, the types of the other species are intermediate in form and exhibit an almost complete transition from the one to the other of the two species mentioned.

The present specimen consists of numuerous long slender branches, not exceeding 3 mm. in thickness and nowhere showing sign of the conspicuous terminal swellings evidenced in THIELE's sponges. Further, although the smaller tylostyli have much the same proportions as in THIELE's specimens, the larger seldom exceed. .8 mm. in length and .01 mm. in thickness, whereas in the Japanese forms they range from .8 to 1.9 mm. long and from .02 to .05 mm. thick. It has, however, the characteristic appearance of the surface. (pl. VIII, fig. 14). There can be therefore, little doubt that the present specimen is identical with *R. clavata*, as here understood.

Distribution. — Japan.

Genus APLYSINOPSIS LENDENFELD.

Genolectotype. — *A. elegans* LENDENFELD. 1889, p. 379, pl. xxvii, fig. 5, pl. xxxiv, figs. 8, 11.

Diagnosis. — Spongiidae with skeleton of pithed fibres forming a quadratic mesh; main fibres regular, usually cored with sand; secondary fibres free of inclusions but branching, and anastomosing to form an irregular network of fibres between main or ascending fibres.

8. **Aplysinopsis lobosa,** sp. n.

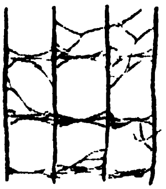
(Pl. VIII, fig. 15; text-figs. 5, 6).

Holotype. — Spec. No. 4. In the collection of the Biological Institute, Tôhoku Imperial University.

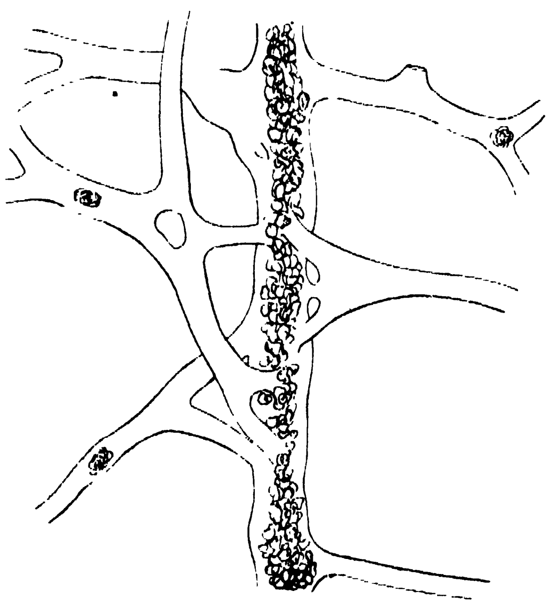
Occurrence. — Aniwa Bay, off Enoura; depth unknown; sandy mud; October, 1929.

Diagnosis. — Sponge massively lobose; surface characters unknown; skeleton a coarse reticulation of fibres of quadratic mesh; primary fibre stout, cored with sand, running in general direction from base of sponge to surface; secondary fibres irregular, with tendency to anastomose and branch in all directions, but generally without foreign inclusions.

Remarks. — The single specimen, although preserved in spirit, was dead long before capture, and retains no trace of the soft tissues. Under ordinary circumstances it would therefore have been doubtful whether to make it a type of a new species. Hitherto only five species of *Aplysinopsis* have been described, *A. elegans*, *A. pedunculata* and *A. digitata*, all of LENDENFELD, and *A. massa* and *A. tuberosa* of SZYMANSKI. The first three, all of which come from New South Wales, Australia, appear to belong to a single species only. Similarly, the two described by SZYMANSKI from Aegina, Mediterranean, appear to be identical forms. We can say therefore that the present species is virtually the third known to us, and for this reason is worth description, even though the material is in a poor state of preservation.



Text-fig. 5. *Aplysinopsis lobosa*, sp. n., to show the arrangement of the skeleton. ×7.



Text-fig. 6. *Aplysinopsis lobosa*, sp. n., showing a portion of the skeleton to show inclusions in the fibres. ×33.

The holotype of *Aplysinopsis lobosa*, sp. n. is a large sponge consisting of 3 lobes, with a small secondary lobe springing from the lowermost of these. It is 13 cms. high, 8 cms. across at the widest part, and 3 cms. through at the thickest point. Although the dermis is gone there can be little doubt that the surface was originally minutely conulose. No oscules are

visible, nor any exhalant openings or canals. The skeleton consists of main fibres, 3 mm. thick cored by sand grains, and the secondary fibres are 2 mm. thick. The secondary fibres are tolerably free from foreign inclusions, except for an occasional sand-grain.

The species differs from *A. elegans* LENDENFELD (syn. *A. pedunculata* et *A. digitata*) and *A. massa* SZYMANSKI (syn. *A. tuberosa*) in form, the former being stipitate with hollow digitate processes and the latter massive and low-growing. In the structure of the skeleton it makes a close approach to the Australian species but in that the fibres contain only a single row of sand-grains. The fibres of *A. massa* are much thinner, main fibres .75 to .112 mm. and secondary fibres .37 to .6 mm. thick, and there does not appear to be any trace of foreign inclusions.

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EXPLANATION OF THE PLATES.

PLATE VII.

Fig. 1. *Semisuberites arctica* CARTER (slightly less than natural size).
Fig. 2. *Semisuberites arctica* CARTER, seen from above and showing the cribriform oscular plates
Figs 3–4. *Uritaia halichondroides*, sp n. ×1/1.
Figs 5 9 *Halichondria panicea* (PALLAS). Figs. 5–6 from specimens from Saghalin, figs. 7–9 from specimens collected around Japan by Mr. INSOLE. ×1/1.
Fig. 10. *Halichondriella corticata* BURTON. ×1/1

PLATE VIII.

Figs. 11–12 *Eumastia sitiens* SCHMIDT. ×1/1.
Fig. 13. *Suberites domuncula* (OLIVI). ×2/3.
Fig. 14. *Rhizaxinella clavata* THIELE. ×1/1.
Fig. 15 *Aplysinopsis lobosa*, sp. n. ×2/3.

M. BURTON: Sponges of South Saghalin.

15

11

14

12

13

M. BURTON: Sponges of South Saghalin.

Report of the Biological Survey of Mutsu Bay.
23. *Rhizopsammia minuta* VAN DER HORST var. *mutsuensis*, nov., an Eupsammid Coral.[1]

BY

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(Institute of Geology and Palaeontology, Tôhoku Imperial University).

(With Plate IX).

(Received Feb. 10, 1932).

The object of this communication is to describe an interesting coral living in Mutsu-Bay; the specimens were collected* by Professor S. HÔZAWA from the littoral zone of a small islet Moura-shima, near the Asamushi Marine Biological Station. We are grateful for his kind offer of the material to our study.

The genus *Rhizopsammia* was established by A. E. VERRILL[2] in his "Notes on the Radiata in the Museum of Yale College, with descriptions of new genera and species," 1867, on a recent species from the Pearl Islands. The generic diagnosis quoted in DUNCAN's[3] "Revision of Madreporaria" is as follows: "Colony low, incrusting, extending by stolon-like expansions of the base, from which buds arise. Corallites cylindrical or nearly so, connected by thin creeping expansions, which have the same texture as the wall. Calices subcircular or elliptical. Septa thin, crowded, a little projecting, arranged in four or five cycles; last cycle well developed, uniting to those of the preceding cycle, which rise up the form of prominent paliform lobes, beyond which the central region of the calice is deep. Columella very porous and its surface papillose. Wall very porous. No epitheca. Costae scarecely distinct, represented by series of rough granules."

In 1922, VAN DER HORST described two new species of the genus based on the materials of the Siboga Expedition, *Rhizopsammia verrillii* and

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* Collected from within a cave situated on the western side of Moura-shima, average depth at low tide about 1.5 m.

1) A contribution from the Marine Biological Station, Asamushi, Aomori-ken. No. 82.

2) A. E. VERRILL: Notes on the Radiata in the Museum of Yale College, with Descriptions of New Genera and Species. Trans. Conn. Ac. of Arts and Sciences, Vol. I, 1866–1871. (Not accecible, cited after P. M. DUNCAN, 1884 and VAN DER HORST, 1922.)

3) P. M. DUNCAN: A Revision of the Families and Genera of the Sclerodermic Zoantharia, Ed. & H., or Madreporaria, 1884, p. 182.

Rhizopsammia minuta, and pointed out that the presence of paliform lobes of septa and the absence of epitheca can not be regarded as diagnostic features of the genus, because paliform lobes are absent in both of his species and epitheca is present, though to a very variable amount, in one of his species (*Rhizopsammia minuta*).

Rhizopsammia is, as stated by VAN DER HORST[1], closely allied with *Balanophyllia* and the budding of its corallites by means of stolon-like basal expansions is peculiar to it and distinctive from the latter genus. It is one of the interesting genera of corals, its species inhabiting the the warm waters of the Pacific and having a wide vertical range; one species (*R. verrilli*) was obtained from the depth 27–278 m. and the other (*R. minuta*) from 36 m. of the Malay Archipelago, while our new variety of the latter species lives on the rocky floor of the littoral region in Mutsu Bay, Northern Japan (L. 40° 55.5′ N.). The genotype is from the Gulf of Panama (Pearl Islands).

Rhizopsammia minuta VAN DER HORST *var. mutsuensis* nov.

(Pl. IX, Figs. 1–3.)

Compare:

1922. *Rhizopsammia minuta* VAN DER HORST, The Madreporaria of the Siboga Expedition, part II, *Eupsammidae*, Siboga-Expeditie, p. 65, Pl. VII, figs. 9–10.

Corallum spreading over the surface of rhyolite blocks and consisting of a large number of corallites connected at the base by stolon-like processes. Corallites fragile, small, 5 mm. or less in diameter, cylindrical or sometimes slightly contracted near the base, projecting at most 8 mm. above the base, usually a few millimeters apart and sometimes almost in contact. Stolon-like expansions 2–4 mm. broad and distinctly costated, costae being continuous with those on the lateral surface of the corallites. Calice circular, as broad as the corallites; 4 mm. deep and surrounded by vertical inner edges of septa. Small or young corallites usually covered by a dense epitheca, with annular rugose lines, from the base to the very margin of calice; sometimes worn out and then exposing the vertically costated lateral surface of wall; epitheca mostly lose in the larger corallites. Wall perforated, pores lying within vertical furrows in alternation with costae. Costae somewhat elevated, rounded and minutely vermiculated. Septa 4 cycles complete in large calices, thin and subequal in thickness.

[1] VAN DER HORST: The Madreporaria of the Siboga Expedition, Pt. II, *Eupsammidae*, Siboga-Expeditie, Monographe XVI a, p. 64, 1922.

Septa of the first and second cycles slightly exsert near the calicular margin where their edge is semicircular in outline, short except on the base of the bottom of calice where they extend to a well developed spongy columella, subcircular in cross section and 1 × 1.5 mm. broad. Septa of the third cycle short and those of the fourth cycle uniting in pairs in front of the former at the base of the calice. All the septa are finely dentated on the edge, densely granulated on the lateral surfaces, and perforated, pores being especially numerous near the wall. In the smaller corallites the septa of the second cycle do not extend to the columella. No paliform lobes.

The present material agrees fairly well with the typical form of *Rhizopsammia minuta* VAN DER HORST[1] from the Roma Islands, but some slight differences are appreciable between them referring to the relative height of the corallites and the surface feature of the stolons. In the typical form, the corallites are more depressed and the stolons are always smooth on their surface instead of being costated. The dimensions of them and other features are compared in the annexed table.

	Breadth of Stolon-like expansion	Height of corallites	Depth of calice	Sculpture of stolon	Septal character
Typical form	1-5 mm.	up to 2 mm.	5 mm.	smooth	4 cycles complete
N. var.	2-4 mm.	up to 8 mm.	4 mm.	ribbed	4 cycles complete

Distinctly costated stolons and septa with spongy margin are features characterizing another species, *Rhizopsammia verrillii* VAN DER HORST[2] from the Malay Archipelagoes; from this the present form is easily distinguished by its smaller and shorter corallites and a different septal arrangement.

Locality: Moura-shima, near Asamushi, province of Mutsu, Japan.

Five living colonies examined, all spreading over rhyolite blocks.

Collector: Prof. S. HÔZAWA.

EXPLANATION OF PLATE IX.

Fig. 1. *Rhizopsammia minuta* VAN DER HORST var. *mutsuensis* nov. (nat. size), with tentacles. (Specimen stored in the Biological Institute.)

Fig. 2. *Rhizopsammia minuta* VAN DER HORST var. *mutsuensis* (nat. size). Type specimen Soft part removed. (Specimen stored in the Palaeontological Institute, Reg. No. 41391).

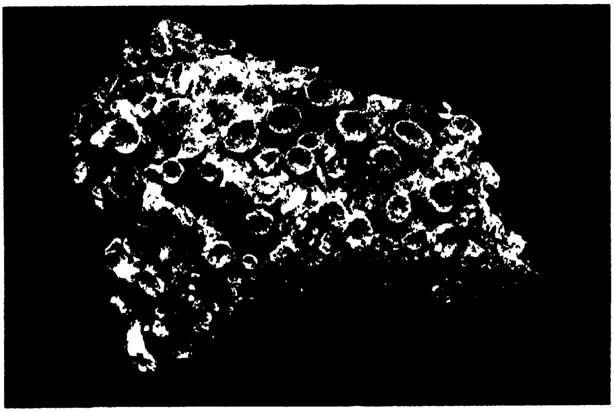
Fig. 3 A part of Fig. 2 enlarged three times.

1) VAN DER HORST, 1922, Op. cit., p. 65, Pl. VII, figs. 9-10.

2) VAN DER HORST, 1922, Op. cit., p. 64, Pl. VIII, figs 1-2.

Fig. 1.

Fig. 2.



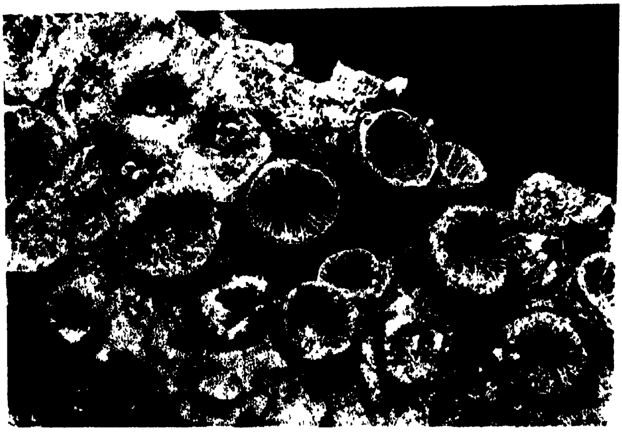


Fig. 3

H. YABE and M. EGUCHI: *Rhizopsammia minuta* VAN DER HORST *var. mutsuensis*, nov.

The Spectral Properties of Haemoglobin in the Holothurians, *Caudina chilensis* (J. MÜLLER) and *Molpadia roretzii* (V. MARENZELLER).[1]

By

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Biological Institute, Tôhoku Imperial University, Sendai, Japan.

(With 5 text-figures and Plate X.)

(Received March 1, 1932).

INTRODUCTION.

Although haemoglobin is rarely found in the blood of most of the invertebrate animals, it occurs in many species of Holothurioidea, so far examined such as the species of *Thyone*, *Cucumaria* and others. In these holothurians, haemoglobin was found in the red corpuscles which are found in the body fluid.

HOWELL (1885) performed various tests of haemoglobin on the laked solution of the corpuscles obtained from the perivisceral cavity of *Thyonella gemmata* (syn. *Thyone gemmata*) and proved that this pigment is identical with haemoglobin chemically and spectroscopically. This haemoglobin solution, however, is different in the coagulation temperature as well as in the precipitation by acetic acid from those shown by most of the vertebrates.

VAN DER HEYDE (1921) studied the pigment in the red corpuscles of *Thyone briareus* found in the Polian vesicle, in the tentacular ampula and in the wall of the water lungs chemically and spectroscopically, and concluded that the pigment under consideration is indentical with haemoglobin.

Recently HOGBEN and VAN DER LINGEN (1927) also noticed the presence of haemoglobin in the red corpuscles of the perivisceral cavity of *Cucumaria frauenfeldi* but found that the absorption spectra of this holothurian haemoglobin differ from the corresponding spectra of the vertebrate haemoglobin.

In some of the investigations other than the holothurian haemoglobin, SORBY (1876) noted that the center of the absorption bands of the *Planorbis* blood differed from those of the vertebrate haemoglobin. He gave the following positional difference in them.

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[1] Contributions from the Marine Biological Station, Asamushi, Aomori-Ken, No. 83.

	The center of the absorption bands.	
	α	β
Vertebrate haemoglobin	581.0	545.0
Planorbis blood	578.0	542.5

SORBY showed at that early period the spectra of haemoglobin of all forms are not alike. This fiinding of SORBY was only recently reexamined by VLÈS (1923) and by ANSON, J. BARCROFT, MIRSKY, and OINUMA (1924).

VLÈS (1923) who measured spectrophotometrically the absorption spectra of haemoglobin from a number of annelids, notably in *Arenicola* compared with the horse and pointed out that there is a difference in the character of the band rather than in its position.

BARCROFT and his collaborators (1924) compared the haemoglobin spectra of various forms of vertebrates and of invertebrates by using the HARTRIDGE reversion spectroscope. They found that the position of the α band and its displacement or the so-called "span" exists between the oxy- and carboxyhaemoglobins.

Thus, these recent works show clearly that the absorption spectra of haemoglobin differs with the species.

Caudina chilensis and *Molpadia roretzii* which I have examined belong to Molpadiidae, and resemble each other closely in their morphological structure. The scarlet coloured pigment is found in the corpuscles which occur in the perivisceral cavity, the blood vessel, the Polian vesicle, and the tentacular ampula.

My observations chiefly dealt with the spectral properties of this scarlet pigment in comparison with the horse haemoglobin. This work was carried out partly at the Marine Biological Station at Asamushi and partly at the Biological Institute in Sendai.

METHOD AND TECHNIQUE.

Caudina chilensis and *Molpadia roretzii*, used in this experiment, are found in Mutsu Bay. The former inhabits the littoral sandy shore at Moura near the Marine Biological Station, while the later is collected by means of dredging the muddy bottom at 30 fathoms, one mile off the station.

The fresh specimens are cut through their body wall, and the perivisceral fluid containing the scarlet coloured corpuscles is collected in a glass vessel. In the freshly dissected specimen, the scarlet colour is seen through the Polian vesicle, the tentacular ampula and the blood vessel. 1/4–1/8 cc.

of blood may be collected from each *Caudina* inserting a needle attached to a small sylinge of 1 cc., into the blood vessel at the portion of the contractile vessel.

The amount of the red corpuscles contained in the perivisceral cavity of *Molpadia* is less than that of *Caudina*. In *Molpadia*, the corpuscles cannot be collected from the blood vessels in the same method as was applied to the *Caudina*.

The fluids thus collected are kept standing for a time, then the corpuscles gradually settle at the bottom leaving a non-coloured or often yellow coloured fluid above. This yellowish colour is easily soluble in the sea water and thus it can be removed from the corpuscles by washing it with the sea water repeatedly. The corpuscles were stirred and centrifugalized repeatedly by addition of a large amount of the filtered sea water till the washed sea water became quite clear. The washed corpuscles were laked with a weak alkaline solution of 0.1% of crystalized sodium carbonate and again centrifugalized and filtered. These laked solutions prepared from both species show a typical absorption spectra of haemoglobin. These holothurian haemoglobin solutions were compared with the jugular venous blood of the horse, which was washed with 0.85% of sodium chloride and then laked with 0.1% of crystalized sodium carbonate.

For the quantitative measurement of the absorption spectra, the KÖNIG-MARTENS's spectrophotometer (Model II) provided with the "Kleine Beleuchtungseinrichtung nach MARTENS", U-shaped glass container (21 mm. length) and SCHULZ's glass were employed. The concentrated filamented lamp (100-watt) was used for the light source. ADAM HILGER's spectrometer and the EASTMAN's panchromatic plate were used for the spectrograms as well as for the determination of the wave length of the maximum and minimum intensities of the haemoglobin spectra, and the BALY's absorption tube as the container of the haemoglobin solution.

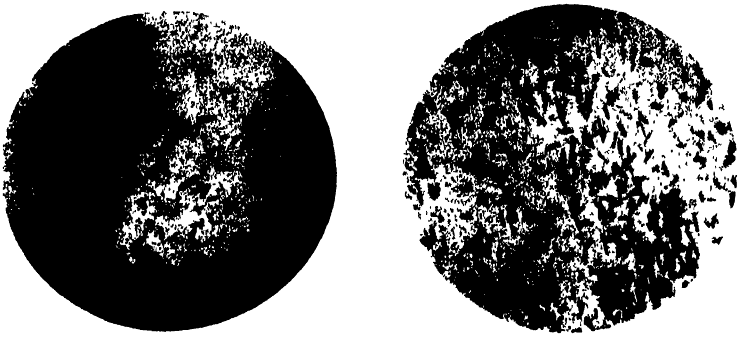
EXPERIMENTAL RESULTS.

1. Preliminary tests for the laked solutions of *Caudina chilensis* and *Molpadia roretzii*.

The preliminary tests were carried out in the laked solutions of the red corpuscles of *Caudina* and of *Molpadia*.

1) The prusian blue tests for iron were positive at the laked solutions of these two species as were already found in *Thyone gemmata* (HOWELL 1885) and *Thyone briareus* (VAN DER HEYDE 1921)

2) TEICHMAN's haemin crystals were microscopically formed with glacial acetic acid and sodium chloride when heated on a slide (Fig. 1, a and b). This reaction was also obtained in *Thyone*.



a b

Fig. 1. Microphotographs of haemin crystals from the holothurian haemoglobin of *Caudina chilensis* (a) and *Molpadia roretzii* (b)

3) VAN DER HEYDE (1921) demonstrated the presence of the proxidase reaction in a few drops of the blood of *Thyone briareus* with bendizine solution and hydrogenproxide. I have also noted that the laked solutions of my own specimens show the similar blue coloured reaction with bendizine solution or guaiac tincture at the presence of hydrogenperoxide. This reaction could be detected even in the coagulated mass of these laked solutions, when heated on the water bath at 100°C.

The centrifugalized and filtered perivisceral fluid, which is free from the red corpuscles, showed also the presence of this reaction but not in the boiled fliud, probably being destroyed by heating its fermentative catalytic activity.

The results of these experiments seem to indicate that the reaction of peroxidase or pseudoperoxidase was due to the presence of iron masked in the red corpuscles. This reaction is also positive in the laked solutions of the corpuscles from the perivisceral cavity, the Polian vesicle, and the tentacular ampula.

1) In these laked solutions the two characteristic absorption bands of oxyhaemoglobin and the single band of the reduced form are visible by the addition of STOKE's reagent or sodium hydrosulphite, but regain the two bands of oxyhaemoglobin by bulbing the air through the solution as will be seen in spectrograms (Plate A-E). The absorption spectra of methaemoglobin, carboxyhaemoglobin and of their derivatives such as

haematin, haemochromogen and haematoporphyrin were seen from these laked solutions similar as those shown by the vertebrate haemoglobin solutions. The spectroscopic observations on oxy- and reduced haemoglobin were already given in *Thyone gemmata* (HOWELL 1885) and in *Thyone briareus* (VAN DER HEYDE 1921).

From the above reactions the scarlet coloured solutions from the red corpuscles of both species possess most properties of haemoglobin. The laked solutions of *Thyone gemmata* and *Cucumaria frauenfeldi* however were reported to be considerably different from the ordinary vertebrate haemoglobin solutions. HOWELL (1885) stated that the coagulation temperature of the laked solution of *Thyone gemmata* was at 58–60°C being contrasted with 70–80°C of the mammalian blood and the laked solution was precipitated by addition of 0.1% of acetic acid. HOGBEN and VAN DER LINGEN (1927) noticed that the laked solution of *Cucumaria frauenfeldi* did not form the compound analogous to sulph-haemoglobin and furthermore ammonium sulphide produced the reduced form, and not by addition of solid sodium thiosulphate.

5) The corpuscles laked with distilled water were coagulated by heating at 59–61°C, similar with that of *Thyone gemmata*, while the coagulation temperature of the horse was at 70–73°C. Both laked solutions are precipitated by addition of the excess of alcohol, and did not separate any coloured substances upon heating or addition of ether, benzine, chloroform, and carbon disulphite, vigorously shaked.

All the above experiments were chiefly carried out with the laked solutions of the corpuscles from the perivisceral cavity.

2. Spectral properties of the holothurian haemoglobin from *Caudina chilensis* and *Molpadia roretzii*.

As was mentioned already, that haemoglobin of *Thyone gemmata* and of *Thyone briareus* shows the two absorption bands in the oxidized form and the single band in the reduced form.

HOGBEN and VAN DER LINGEN (1927) reported from the results of the first spectroscopic measurement of the holothurian haemoglobin, using *Cucumaria frauenfeldi* that the maximum intensity of the oxyhaemoglobin spectra is at 579.0 and 543.0; that of the reduced form, at 558.0, while the two bands α and β of carboxyhaemoglobin are at 573.0 and 538.0 respectively. From these data they showed that the spectra of these haemoglobins were more shifted towards the red end than that of the vertebrate haemoglobin.

Firstly I have made the spectrometric determinations of the laked solutions of the corpuscles from the perivisceral cavity in the two holothurian species, compared with that of the horse and the results are shown in Table I.

TABLE I.

Materials	Oxyhaemoglobin			Reduced haemoglobin (by $Na_2S_2O_4$)
	Maximum intensity of α band	Minimum intensity between α and β band	Maximum intensity of β band	Maximum intensity
Caudina chilensis	579.7	564.0	543.5	561.0
Molpadia roretzii	577.0	561.5	540.7	557.0
Horse	577.0	560.0	541.0	556.0

It is evident from Table I that haemoglobins of the above three different forms differ spectrometrically more or less from one other. The absorption spectra of oxy- and reduced haemoglobin of *Caudina* were found to be remarkably shifted towards the red when compared with those of *Molpadia* and the horse.

Spectrophotometry of holothurian haemoglobin.

The spectrophotometric observations were carried out on the laked solutions of the red corpuscles from the perivisceral cavity, the blood vessel of *Caudina* and from the perivisceral cavity of *Molpadia roretzii*. The sexes were separately observed.

The instrument was carefully calibrated with the known line spectra of the various metals and standardized with a known concentration of potassium bichromate solution before its use. The oxyhaemoglobin spectra were measured under the atmospheric oxygen tension, and the reduced haemoglobin was prepared by addition of a small amount of powdered sodium hydrosulphite to the oxyhaemoglobin solution. The U-shaped container used in the case of reduced haemoglobin was covered with the glass plate and was sealed with vaseline. The spectrophotometric observations were made at 15–20°C.

A. Haemoglobin spectra of *Caudina chilensis*.

The spectrophotometric curves of oxy- and reduced haemoglobin from the perivisceral cavity are shown in Fig. 2 together with those of the horse blood.

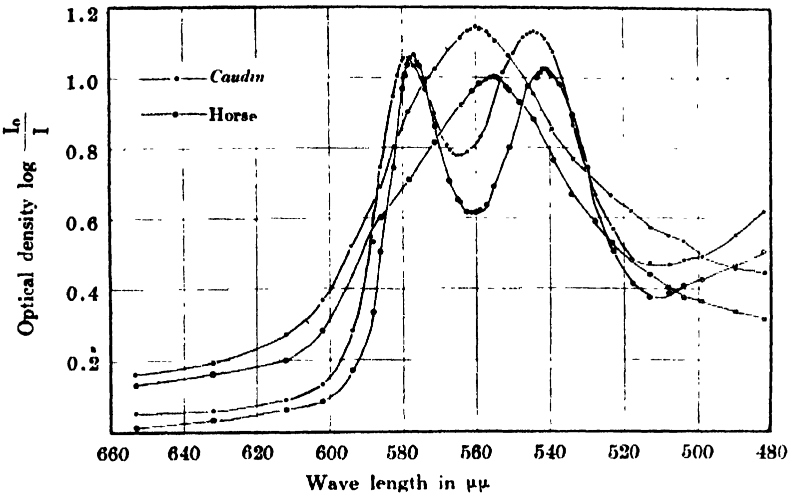


Fig. 2. Spectrophotometric curves of oxy- and reduced haemoglobin of *Caudina chilensis* and the horse. The concentration of these haemoglobin was not determined. (The values from which these curves are drawn in this figure, are given in the Appendix.)

Oxyhaemoglobin: — As seen in Fig. 2 the curves of each haemoglobin spectra of *Caudina* do not exactly coincide with the corresponding curve of the horse, the former being shifted towards the red. And the value of the absorption intensity shown by *Caudina* at the region of 565.0–482.0 is higher than that shown by the horse, specially those nearer the β band.

There are three important characteristics to be noted in the spectrophotometric curves given by oxyhaemoglobin; the maximum intensity of the α band in yellow, the maximum intensity of the β band in green, and the minimum intensity between these maximum intensities. The maximum intensity of the α band is located at 579.5 and that of the β band at 544.2, while the maximum intensity is found at 561.5. Those of the horse are found at 577.0, 542.0, and 560.0, respectively. The positions of these points are distinctly shifted towards the red as compared with those of the horse.

The absorption ratio of $\frac{\alpha}{\beta}$ is found to be 0.92, and that of $\frac{\text{Absorption minimum}}{\beta}$, 0.66, while those of the horse are at 1.03 for $\frac{\alpha}{\beta}$ and 0.62 for $\frac{\text{Absorption minimum}}{\beta}$. The difference of the absorption ratios between

Caudina and the horse shows that in *Caudina* the absorption light in the β band is more than that in the α band and consequently in the horse the absorption of light in the β band is much weaker than in the α band.

Reduced haemoglobin: — The curves of reduced haemoglobin from the perivisceral cavity and the horse are shown in Fig. 2. In this case, the single band can be seen in *Caudina* and the horse. The general character of two curves are quite similar. The position of the maximum intensity of each band is at 560.0 in *Caudina* and at 555.5 in the horse. The shifting of this band is greater in *Caudina*, compared with the corresponding band of the horse as was already noted in the case of oxyhaemoglobin.

KAWAMOTO (1927) stated in his paper concerning the morphology of the corpuscles of *Caudina chilensis* that the various forms of the red and brown coloured, and non-coloured corpuscles were found, although the number of corpuscles varied with the blood vessel, the water canal, and the perivisceral cavity. Furthermore, besides the large number of the red corpuscles, he described the presence of a small amount of the brown coloured corpuscles at the perivisceral cavity, but not in the blood vessel.

The maximum and minimum intensities, and their absorption ratios of the haemoglobin solution from the blood vessel were compared with those of the haemoglobin from the perivisceral cavity according to sex. These results are given in Table II and the graphical representation is also shown in Fig. 3.

TABLE II.

Haemoglobin from	Case of experiments	Oxyhaemoglobin					Reduced haemoglobin (by $Na_2S_2O_4$)
		Maximum intensity of α band	Minimum intensity between α and β bands	Maximum intensity of β band	Absorption ratio of $\frac{\alpha}{\beta}$	Absorption ratio of $\frac{\text{Absorption min.}}{\beta}$	Maximum intensity
Perivisceral cavity. ♀	8 (each individual)	579.5	564.5	544.2	0.91 ± 0.03	0.66 ± 0.03	560.0
Perivisceral cavity. ♂	9 (each individual)	579.5	564.5	544.2	0.92 ± 0.03	0.66 ± 0.02	560.0
Blood vessel ♀	1 (3 individuals)	579.5	564.5	544.2	0.92	0.68	560.0
Blood vessel ♂	1 (4 individuals)	579.5	564.5	544.2	0.92	0.67	560.0
Horse	9 (each individual)	577.0	560.0	542.0	1.03 ± 0.02	0.61 ± 0.02	555.5

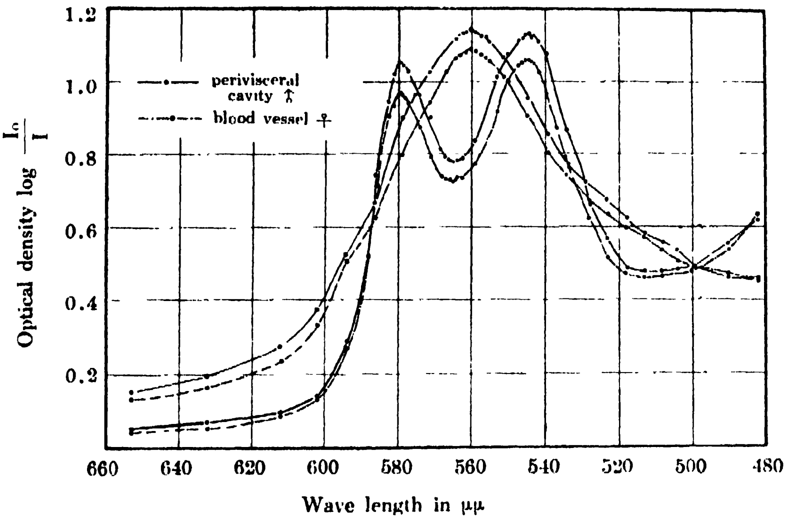


Fig. 3. Spectrophotometric curves of oxy- and reduced haemoglobin from the perivisceral cavity and the blood vessel in *Caudina chilensis*. The concentration of these haemoglobin was not determined. (The values from which these curves are drawn in this figure, are given in the Appendix.)

As seen in both Table II and Fig. 3, the spectral properties of the holothurian haemoglobin solution from both the blood vessel and perivisceral cavity, show similar results with each other, irrespective of the sexes.

B. Haemoglobin spectra of *Molpadia roretzii*.

In my previous observations, it was shown that in both the α and β bands of *Molpadia roretzii* the positions of the maximum intensities are nearly identical with the horse, but that of the minimum intensity between these bands showed a slight shift towards the red, as compared with the horse.

The spectrophotometric curves of oxy- and reduced haemoglobin of *Molpadia* and of the horse are shown in Fig. 4.

Oxyhaemoglobin : — The maximum intensities of oxyhaemoglobin spectra of *Molpadia* are located at 577.0 in the α band and at 541.5 in the β band similar to those in the horse. The minimum intensity between these two bands is located at 562.0 showing a slight shift towards the red as compared with that in the horse.

The absorption ratios of the three chief characteristics of *Molpadia*

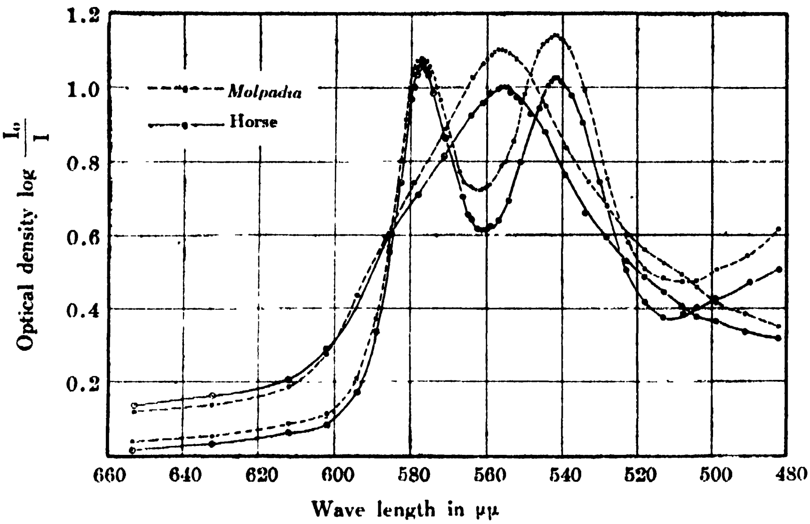


Fig. 4. Spectrophotometric curves of oxy- and reduced haemoglobin of *Molpadia roretzii* and the horse. The concentration of these haemoglobin was not determined. (The values from which these curves are drawn in this figure, are given in the Appendix.)

are seen to be 0.91 in the case of $\frac{\alpha}{\beta}$ and 0.64 in the case of $\frac{\text{Absorption } \beta}{\text{minimum}}$. From these results, the oxyhaemoglobin spectra of *Molpadia* are noticiably different from the horse haemoglobin, having the high value of the absorption intensity at the region of 565.0–482.0 or specially nearer the β band.

Reduced haemoglobin: — The general appearance of the reduced haemoglobin resembles that of the horse and also that of *Caudina*, having a single band. The maximum intensity of this band is located at 557.0, and lies between the corresponding band of *Caudina* and the horse. All the data on oxy- and reduced haemoglobin from the different individuals are given in Table III.

Comparing the general character of the curves, oxyhaemoglobin of *Molpadia* resembles that of *Caudina*, both showing the high value of the β band, though the minimum intensity at green between the α and β bands is lower than in *Caudina*.

The positions of the maximum and minimum intensities of oxy- and reduced haemoglobin are noticiably shifted towards the violet end as compared with those in *Caudina* as will be seen in Fig. 5.

TABLE III.

Haemoglobin from the perivisceral cavity.	Oxyhaemoglobin					Reduced haemoglobin (by $Na_2S_2O_4$)
	Maximum intensity of α band	Minimum intensity between α and β bands	Maximum intensity of α band	Absorption ratio of $\frac{\alpha}{\beta}$	Absorption ratio of $\frac{\text{Absorption min.}}{\beta}$	Maximum intensity
Molpadia roretzii ♂	577.0	562.0	541.5	0.94	0.63	557.0
,,	577.0	561.5	541.5	0.92	0.63	557.0
,, ♀	577.0	562.0	541.5	0.92	0.64	557.0
,,	577.0	562.0	541.0	0.90	0.65	557.0
,,	577.0	562.0	541.5	0.89	0.64	558.0
Mean	577.0	562.0	541.5	0.91±0.03	0.64±0.02	557.0
Horse	577.0	560.0	542.0	1.03±0.02	0.61±0.02	555.5

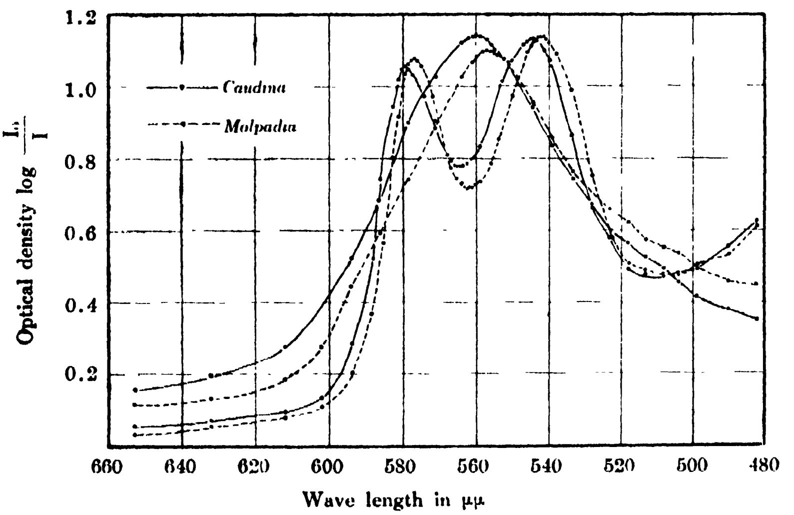


Fig. 5. Spectrophotometric curves of oxy- and reduced haemoglobin from the perivisceral cavity of *Caudina chilensis* and *Molpadia roretzii*. The concentration of these haemoglobin was not determined. (The values from which these curves are drawn, are given in the Appendix.)

From the above experiments on holothurian haemoglobins of *Caudina chilensis* and of *Molpadia roretzii*, it is concluded that haemoglobin shows difference according to species which are closely related to each other that is the two species belonging to the same family.

CONCUSION.

The scarlet coloured pigments contained in the red corpuscles of *Caudina chilensis* and *Molpadia roretzii* were identified to be haemoglobin but they differ from the mammalian haemoglobin (horse) at the coagulation temperature. They do show some small but definite differences from each other when two closely ralated species of holothurian are compared spectroscopically.

The spectrophotometric results obtained from *Caudina* and *Molpadia*, and haemoglobins of the various sources which were studied by several former investigators are tabulated in Table IV.

Comparing these data of *Caudina* and *Molpadia* and other haemoglobins from the vertebrates and invertebrates, in the spectra of oxyhaemoglobin the position of the maximum and minimum intensities of *Caudina chilensis* is found to be shifted extremely towards the red end as well as in the case of *Cucumaria frauenfeldi* (HOGBEN and VAN DER LINGEN, 1927), but those of *Molpadia roretzii* do not show any noticiable difference though the minimum intensity at 562.0 is slightly shifted towards the red end.

The absorption ratio of $\frac{\alpha}{\beta}$ is 0.92 for *Caudina* and is 0.91 for *Molpadia* and thus remarkably differs when compared with 1.03 for the horse and 0.98–1.04 for the other mammarian oxyhaemoglobin (HÁRI 1917, VLÈS 1921, KENNEDY 1926) and also differs from the invertebtate haemoglobin of *Urechis caupo* 0.99 for $\frac{\alpha}{\beta}$ (REDFIELD and FLORKIN 1931). On the other hand, the $\frac{\text{Absorption minimum}}{\beta}$ is 0.66 for *Caudina* and 0.64 for *Molpadia* indicating a higher value than that of the other forms of haemoglobins.

Such a form of oxyhaemoglobin in which the β band shows the higher value of the absorption intensity of light, has not yet been reported in the case of the vertebrates, though it is reported in *Arenicola* and *Marphysa* by VLÈS (1923) among invertebrates.

The reduced haemoglobin spectra of both holothurian species consist of a single band, and the position of the maximum intensity of these haemo-

TABLE IV.

Haemoglobin from	Occurrence	Oxyhaemoglobin: Maximum intensity of α band	Oxyhaemoglobin: Minimum intensity between α and β band	Oxyhaemoglobin: Maximum intensity of β band	Oxyhaemoglobin: Absorption ratio of $\frac{\alpha}{\beta}$	Oxyhaemoglobin: Absorption ratio of $\frac{\text{Absorption min.}}{\beta}$	Reduced haemoglobin: Maximum intensity	Investigator
Arenicola piscatorum	in solution	576.0	560.0	540.4	0.95	0.63	563.0 and **550.0**	VLÈS (1923)
Marphysa sanguinea	in solution	578.0	—	540.0	0.88	—	571.0 and **551.0**	„ („)
Urechis caupo	in corpuscles	577.0 *	561.0 *	542.0 *	0.99 *	0.63 *	556.0	REDFIELD and FLORKIN (1931)
Cucumaria frauenfeldi	in corpuscles	579.0	—	543.0	—	—	558.0	HOGBEN and VAN DER LINGEN (1927)
Caudina chilensis	in corpuscles	579.5	564.5	544.2	0.92	0.66	560.0	Writer
Molpadia roretzii	in corpuscles	577.0	562.0	541.5	0.91	0.64	557.0	„
Horse and dog	in corpuscles (crystalized)	575.6	558.1	540.4	0.98 *	0.60 *	—	HÁRI (1917)
Horse	in corpuscles (crystalized)	578.5	562.5	543.5	1.03	0.63	556.0	VLÈS (1921)
Horse	in corpuscles (venous blood)	577.0	560.0	542.0	1.03	0.62	555.5	Writer
Dog	in corpuscles (venous blood)	575.5 *	560.0 *	540.0 *	1.04 *	0.61 *	—	KENNEDY (1926)
Human blood	in corpuscles (venous blood)	575.5 *	561.5 *	540.0 *	1.04 *	0.60 *	—	„ („)
Donky	in corpuscles (venous blood)	576.0 *	560.0 *	540.0 *	1.03 *	0.58 *	—	„ („)

* Estimated from the data published by the investigators. Heavy numerical letters indicate the positions of secondary or accessory band.

globins is considerably more shifted towards the red end, specially in *Caudina* in which is shown remarkable shifting towards the red end as compared with the reduced form shown by vertebrate and invertebrate. In such reduced haemoglobin, the occurrence of the secondary or accessory band which was noted notably in *Arenicola* and *Marphysa* by VLÈS (1923), cannot be found in these holothurian haemoglobins, but resembles the echurian haemoglobin of *Urechis caupo* (REDFIELD and FLORKIN 1931) or the ordinary mammalian haemoglobin.

In conclusion, I wish to express my sincere thanks to Prof. S. HATAI for his valuable suggestions and critisisms during the whole course of this work. I am also grateful to Asist. Prof. S. NOMURA of our Institute of Biology and to Prof. H. OHSHIMA of the Zoological Institute of the Department of Agriculture at the Kyushu Imperial University, for their kind advice and help, and to the members of the Marine Biological Station at Asamushi for the abundant supply of the materials. My thanks are also due to Prof. J. ÓKUBO of the Physical Institute, for the help in the technique in spectroscopy.

SUMMARY.

1. *Caudina chilensis* and *Molpadia roretzii* possess the scarlet coloured pigment which is found in the red corpuscles in the perivisceral cavity, the Polian vesicle, the tentacular ampula and the blood vessel.

2. The scarlet coloured pigment of these species is identified to be haemoglobin since it shows most properties of the mammalian haemoglobin (horse) though it differs at the coagulation temperature.

3. The spectra of oxyhaemoglobin of these species do not only show the positional difference of the two typical bands, but also show that the value of the absorption intensity of the β band is higher than that of the horse haemoglobin. In the reduced form, a single band can be observed in these haemoglobin solutions, as in the case of the horse haemoglobin, but they show a disparity characteristic to each species. In both oxy- and reduced haemoglobin of *Caudina*, the positions of the absorption bands are more shifted towards the red than those of *Molpadia*, but this difference is very slight when compared with the similar difference found between the holothurian species and vertebrates.

4. It is concluded that even in closely related holothurian species haemoglobins differ from one other.

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APPENDIX.

Spectrophotometric values in the table, from which the curves of Fig. 1, 2, 3, and 4 were constructed, are shown as optical density, log $\frac{I_0}{I}$.

Wave length in mμ	*Caudina chilensis*				*Molpadia roretzii*		Horse blood	
	Perivisceral cavity ♂		Blood vessel ♀		Perivisceral cavity ♂			
	Oxy-haemoglobin	Reduced haemoglobin	Oxy-haemoglobin	Reduced haemoglobin	Oxy-haemoglobin	Reduced haemoglobin	Oxy-haemoglobin	Reduced haemoglobin
653.0	0.055	0.159	0.041	0.131	0.031	0.119	0.013	0.133
632.0	0.063	0.199	0.052	0.168	0.058	0.136	0.039	0.164
612.0	0.091	0.277	0.091	0.236	0.080	0.183	0.064	0.204
602.0	0.136	0.374	0.131	0.329	0.110	0.277	0.087	0.282
594.0	0.283	0.524	0.280	0.507	0.205	0.436	0.176	
588.0	0.524	—	0.522		0.370	—	0.332	—
586.0	0.745	0.689	0.717	0.623	0.570	0.599	0.506	0.600
582.5	0.944	—	0.902	—	0.800	—	0.742	—
581.0	1.021	—	0.930	—	0.913	—	—	—
580.0	1.048	—	0.961	—	—	—	0.970	—
579.5	1.055	—	0.973	—	1.035	—	1.010	—
578.5	1.049	0.900	0.967	0.798	1.063	0.744	1.037	0.709
578.0	—	—	—	—	—	—	1.055	—
577.0	1.031	—	0.940	—	1.078	—	1.064	—
576.0	—		—	—	1.072	—	1.049	—
575.0	—	—	—	—	1.056	—	1.033	—
574.0	0.964	—	0.876	—	—	—	0.988	—
572.0	—	—	—	—	0.969	—	—	—
571.0	0.900	1.026	0.791	0.943	—	0.886	0.861	0.817
568.0	0.811	—	0.748	—	—	—		—
567.0	0.796	—	0.734	1.023	0.772	—	0.702	—
566.0	0.786	—	0.731	—	—	—	—	—
565.5	0.782	—	0.727	—	0.743	—	0.650	—
565.0	0.781	—	0.731	—	—	—	—	—
564.0	0.781	1.118	0.735	1.068	0.731	1.023	0.645	0.923
563.5	—	—	—	—	0.720	—	—	—
562.5	0.787	1.133	0.738	1.070	0.718	—	0.618	—
562.0	—	—	—	—	0.720	—	—	—
561.0	—	1.136	—	1.080	0.721	1.064	0.616	0.964
560.5	—	—	—	—	0.724	—	0.615	—
560.0	—	1.142	—	1.091	0.729	1.081	0.617	—
559.0	0.839	1.136	0.772	1.083	0.737	1.087	0.622	0.987
558.0	—	—	—	—	—	1.095	—	—
557.0	—	1.132	—	1.077	—	1.098	0.639	—
556.5	—	—	—	—	—	1.097	—	0.995
556.0	—	1.121	—	1.066	0.788	—	—	1.004
555.5	—	—	—	—	—	1.093	—	1.004
555.0	—	1.103	—	1.054	—	—	0.690	1.003
554.0	—	—	—	—	—	1.900	—	0.987
553.0	1.015	—	0.920	—	0.852	—	—	—
552.0	—	—	—	—	—	1.074	—	0.974
551.0	—	1.069	—	1.013	—	—	0.800	0.960
550.0	1.073	—	1.001	—	0.980	—	—	—
548.5	—	—	—	—	—	—	—	0.931

Wave length in μμ	*Caudina chilensis* Perivisceral cavity ♂ Oxy-haemoglobin	*Caudina chilensis* Perivisceral cavity ♂ Reduced haemoglobin	*Caudina chilensis* Blood vessel ♀ Oxy-haemoglobin	*Caudina chilensis* Blood vessel ♀ Reduced haemoglobin	*Molpadia roretzii* Perivisceral cavity ♂ Oxy-haemoglobin	*Molpadia roretzii* Perivisceral cavity ♂ Reduced haemoglobin	Horse blood Oxy-haemoglobin	Horse blood Reduced haemoglobin
548.0	—	—	—	—	—	1.030	—	—
547.0	1.114	—	1.017	—	—	—	—	—
546.0	1.121	—	1.054	—	1.035	—	0.975	—
545.0	1.130	0.953	1.058	0.905	1.111	0.950	—	0.881
544.2	1.132	—	1.057	—	—	—	—	—
543.5	1.126	—	1.050	—	1.123	—	1.000	—
543.0	—	—	—	—	1.135	—	—	—
542.5	—	—	—	—	1.136	—	1.016	—
542.0	—	—	—	—	—	—	1.024	—
541.5	1.010	—	1.020		1.137	—	1.025	—
541.0	—	—	—	—	1.131	—	1.017.	—
540.5	—	—	—	—	1.121	—	1.012	—
539.5	1.074	0.855	0.965	0.803	—	0.838	—	0.767
538.0	—	—	—	—	1.031	—	0.983	—
537.5	—	—	0.873	—	—	—	—	—
534.5	—	—	—	—	0.993		0.906	—
534.0	0.867	0.770	0.796	0.739	—	0.747	—	0.661
530.0	—	—	—	—	—	—	0.745	—
528.0	0.662	—	0.636	0.679	0.751	0.675	—	0.593
523.0	0.570	0.663	0.514	0.637	0.583	0.599	0.505	0.521
518.0	0.488	0.625	0.475	0.595	0.505	0.563	0.415	0.487
513.0	0.479	0.574	0.460	0.570	0.487	0.527	0.377	0.447
508.0	0.470	0.552	0.465	0.535	0.474	0.494	0.385	0.406
504.0	0.484	0.540	0.470	0.507	0.475	0.453	0.410	0.374
499.0	0.492	0.494	0.537	0.494	0.504	0.420	0.423	0.365
490.0	0.557	0.460	0.539	0.478	0.533	0.384	0.468	0.332
482.0	0.625	0.450	0.635	0.457	0.616	0.351	0.505	0.316

EXPLANATION OF PLATE X.

A–E. Spectrograms of haemoglobin spectra from the corpuscles in the perivisceral cavity of *Caudina chilensis* and *Molpadia roretzii*. The line spectra of mercury are shown as the standard.

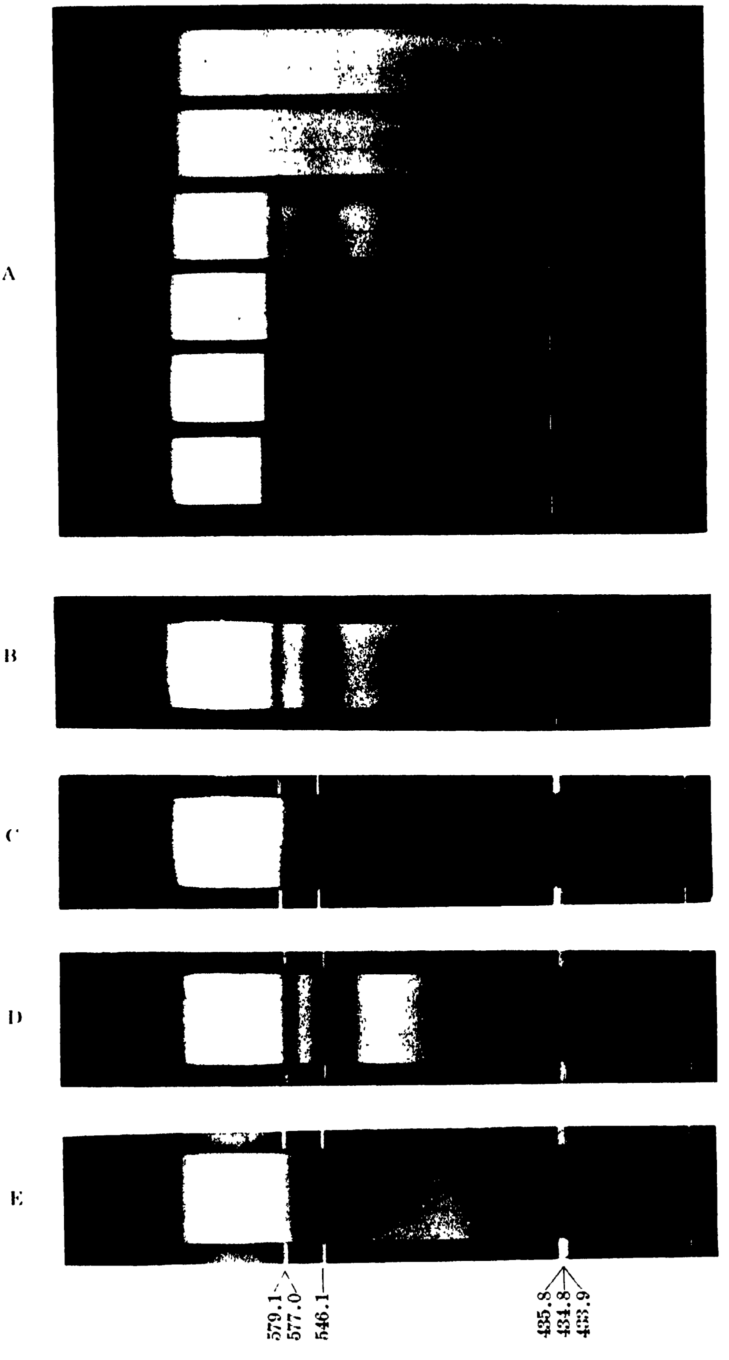
A. General appearance of oxyhaemoglobin spectra of *Caudina chilensis* at the various concentrations.

B. Absorption spectra of oxyhaemoglobin in *Caudina chilensis*.

C. Absorption spectra of reduced haemoglobin in *Caudina chilensis*.

D. Absorption spectra of oxyhaemoglobin in *Molpadia roretzii*.

E. Absorption spectra of reduced haemoglobin in *Molpadia roretzii*.


A
B
C
D
E
579.1
577.0
546.1
435.8
434.8
433.9

On the Osmoregulation of the Blood of Several Marine and Fresh Water Molluscs.[1]

I. The Japanese Oyster, *Ostrea circumpicta* PILS.

By

MASAYASU YAZAKI.

(With 3 Text-figures.)

(Received March 3, 1932).

The present investigation deals with the regulatory ability of the oyster to fresh water and is the continuation of the work which has been carried out a few years ago (YAZAKI, '29). The similar observations were extended to different molluscs other than the oyster and the results are reported in the present paper.

1) *Changes noted when oysters were transferred from sea water to fresh water.*

It is a well known fact that most marine animals cannot live long in fresh water, though some may survive in distilled water containing 1 per cent. of sea water.

DAKIN ('09) reported that "an oyster placed in fresh water might live for some time without any change taking place in the osmotic concentration of the blood. This, however, is simply due to the animal closing the shell valves and completly shutting out the external medium from any contact with the body."

DUVAL ('25, '28) studied the Δ of the blood in some marine invertebrates which were transferred into fresh water, but did not report the changes that led to results.

So far as my own observations go that when oysters in fresh water close their shell valves perfectly, avoiding the contact with fresh water and in that manner maintain the original blood concentration for five or six days. Some oysters kept in fresh water may often survive as long as a month, though the majority of them endure only about 20 days at 19–20°C. and the regulation of the osmotic concentration of the blood is maintained chiefly by closing their shells. But the closing of shells is not permanently

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[1] Contributions from the Marine Biological Station, Asamushi. No. 84.

complete and after a while some amount of fresh water naturally enters the body. Then, the blood concentration falls gradually and proportionately with the length of time in contact with the fresh water. The limitation of dilution is about 65 per cent. of the original concentration when kept in fresh water contrasted with about 70 per cent. dilution when the oyster was kept in half diluted sea water as I have already reported (YAZAKI, '29). It was also noted that when the oyster shell was perforated, the fresh water enters at once and the blood concentration decreases rapidly to about 60 per cent. of the original concentration but remains in this condition for several days.

When, however, the concentration falls to about 65 per cent. of the original, it becomes stationary and such "a relatively stable phase" remains for days. To what extent and how rapidly the concentration of blood is diluted depends on the nature of media used, for instances, at about the 8th day after immersion in fresh water the blood shows 65 per cent. dilution, while those immersed in half diluted sea water shows 70 per cent. of the original concentration at the 10th day.

It seems clear from the above, that oysters either in fresh water or in diluted sea water are able, for some length of time, to prevent the dilution of the blood concentration by the powerful closure of the shells, but sooner or later it begins to decrease gradually and ultimately becomes about 65 per cent. of the original concentration, beyond which its rate of decrease becomes very slow showing "stable phase" for many days, but shows an abruptly rapid fall if the oyster was kept still longer. This

TABLE 1.

Time of immersion in fresh water	Blood Δ with perfect shells	Blood Δ with perforated shells
0 hr.	1.970	1.970
6 hrs.	—	1.500
12 ,,	—	1.472
1 day	1.970	1.302
2 days	1.970	1.261
3 ,,	1.932	1.201
4 ,,	1.921	1.184
5 ,,	1.893	1.167
6 ,,	1.871	1.124
7 ,,	1 774	1.048
8 ,,	1.517	0.946
9 ,,	1.359	0.828
10 ,,	1.320	
11 ,,	1.281	

(continued)

Time of immersion in fresh water	Blood Δ with perfect shells	Blood Δ with perforated shells
12 days	1.307	
13 ,,	1.264	
14 ,,	1.279	
15 ,,	1.274	
16 ,,	1.268	
17 ,,	1.275	
18 ,,	1.271	
19 ,,	1.261	
20 ,,	1.232	
21 ,,	1.223	
22 ,,	1.200	
23 ,,	1.166	
24 ,,	1.162	
25 ,,	1.122	

(These data are given from 5 oysters used.)

rapid fall of the concentration is coincident with the loss of the power of defending the body against the injurious action of fresh water. The results of this experiment are given in Table 1 and Fig. 1.

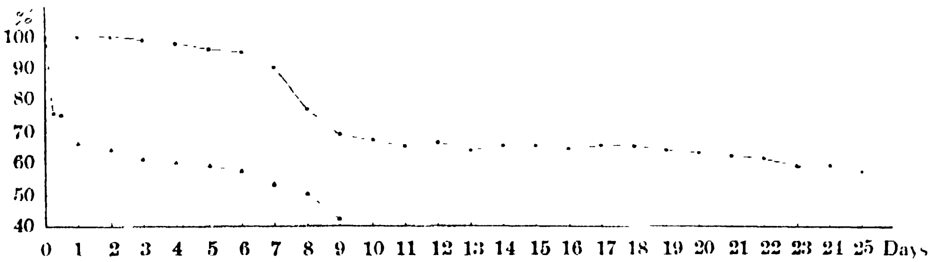

Fig. 1. Curves of the freezing point depression of the oysters in fresh water. The immersion period in days is plotted as the abscissae, and the freezing point (%) as the ordinates. —•— Oysters with perfect shell valves. • Oysters with perforated shell valves.

The blood of a dying oyster immersed in fresh water for a long period gives the value of Δ from about 1.20–1.10. It is usually the case that, when the blood concentration reaches to about 55 per cent. (Δ = 1.100) of the original concentration, the oyster body decays sooner or later, but even at this stage of Δ the recovery is quick if the oyster is returned to normal sea water. In so far as the value of Δ is concerned, the range in which the oyster maintains life, is very narrowly limited; that is between 1.00–1.20.

The values of the blood Δ of dying oysters are shown in Fig. 2.

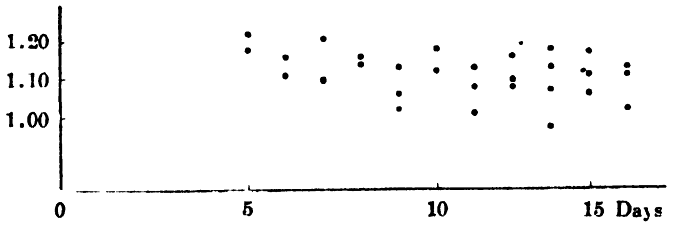

Fig. 2. Blood Δ of dying oysters with shell valves just beginning to open and the stimulus produced hardly any reaction fall within the range 1.00-1.20.

The similar observations which were carried out with some other species of marine molluscs are given in Table 2.

TABLE 2.

Marine species	Original blood Δ	Blood Δ' of dying animals in fresh water	Ratio Δ'/Δ	Fresh water Δ''	Δ'-Δ''
Ostrea circumpicta	1.950	1.10	0.56	0.025	1.075
Anadara inflata	1.973	1.50	0.76	,,	1.475
Pecten yesoensis	1.965	1.40	0.71	,,	1.375
Mytilus hirsutus	1.987	1.30	6.65	,,	1.275

The values of the ratios indicate that different animals show different degrees of resistance when kept in fresh water. Furthermore, the fact that the differences between the values of the blood Δ' and of the fresh water Δ'' are practically identical with those given by the bloods of respective dying animals, indicates at once that the blood of the former cannot become much more diluted without exposing itself to the death point.

The regulation of the osmotic pressure is similar whether the shells was perforated or intact. However, in the oyster with the perforated shell the water contacts itself immediately with the body, so the blood changes much faster as will be seen from Table 3. In Table 3 each given value is the average taken from 5 oysters. In the first 24 hours the decreasing rate of Δ is very rapid. During this period the osmotic process may follow the general principle of diffusion and produces a fall from 100 to about 60 per cent. of the original osmotic concentration. After this stage was reached further dilution of the blood concentration becomes very slow for a considerably long period indicating the probable presence of regulatory or defending capacity in the animal body against an adverse situation. I have, however, already shown (YAZAKI, '29) that

the oysters are unable to maintain osmotic equilibrium much longer even when kept in one half diluted sea water.

The curves of Fig. 1 show that the adjusting period of a certain concentration for the first several days in fresh water is practically identical with or without holes in the shells of oysters. The rate of reduction (t) of the concentration (c), when the shells are intact, may be expressed in the following equation:

$$t=7.6+\mathit{Cot}\,4.5^\circ(c-62)\ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

If we now substitute $6t+3$ and $2.8c-122.5$ for t and c respectively, in order to compare the two curves directly for any given point, we obtain:

$$t=0.767+0.167\,\mathit{Cot}\,4.5^\circ(2.9c-184.5)\ \ldots\ldots\ldots\ldots\ldots(2)$$

This equation (2) expresses satisfactorily the rate of reduction of the perforated shell. As will be seen from Table 3 the observed values of t and c agree well with those computed.

TABLE 3.

Time in day	Blood Δ (%) of perfect shells	$t=7.6+\mathit{Cot}\,4.5^\circ(c-62)$ (Calculated)	Blood Δ (%) of perforated shells	$t=0.767+0.167\,\mathit{Cot}\,4.5^\circ(2.9c-184.5)$ (Calculated)
0	100	100	100	
$\frac{1}{4}$	—	—	76	
$\frac{1}{2}$	—	—	75	75
1	100	100.1	66	66.3
$1\frac{1}{2}$	—	—	—	64.6
2	100	99.7	64	64.2
$2\frac{1}{2}$	—	--	—	64
3	99	99.3	61	
4	98	98.3	60	
5	96	97.3	59	
6	95	95	57	
7	90	88.9	53	
8	77	77.2	48	
9	69	69.8	42	
10	67	67		
11	65	65.6		
12	66	64.8		
13	64	64.3		
14	65	64		
15	65	63.7		

(These data are given from 5 oysters used.)

2) *The effect of fresh water on some physico-chemical properties of the blood.*

Concerning the effect of NaCl on the freezing point depression of oyster blood, I foundal ready (YAZAKI, '29) that NaCl exerts 93 per cent.

of the total osmotic pressure in the blood; showing, therefore, that the osmotic pressure of the blood largely depends upon NaCl which is dissolved.

It is a well known fact that the CO_2 in blood exists in two forms, partly in solution and partly united with the sodium. The quantity of CO_2 gas contained in the blood of marine molluscs is slight when compared with the fresh water ones. KOKUBO ('29) found that the CO_2 gas content of oyster blood is 4.22 vol. %, (range from 3.06 to 5.11 vol. %). I have, however, found that the CO_2 content of fresh water molluscs is very large; for instance in *Anodonta*, about 30 vol. % or more. The relative quantities of the fixed CO_2 and of the free CO_2 at each state are usually given by HENDERSON-HASSELBALCH's equation

$$pH = pk + log \frac{\text{fixed-}CO_2}{\text{free-}CO_2}$$

where *pk* is constant. The molluscs, in general, give the value of 6.1 for *pk*.

The *pH* of the blood in all the specimens used gave value 7.2 by the colorimetric method. This value 7.2 accords well with that found from *Ostrea gigas* living in Matsushima Bay near Sendai City.

According to KOKUBO ('29) the *pH* of the oyster blood normally ranges from 7.8 to 6.74. I have, however, found no such wider range of variation, but *pH* 7.2 in the oyster kept in the normal sea water, changes only to 7.4 even when the oyster was kept 2 weeks in fresh water. The values of the fixed CO_2 and of the free CO_2 were calculated from HENDERSON-HASELBALCH's equation based on the data obtained from my own experiments.

	pH	fixed-CO_2 (%)	free-CO_2 (%)
Oyster in normal sea water	7.2	93	7
Oyster kept 9 days in fresh water	7.3	94	6
Oyster kept 2 weeks in fresh water	7.4	95	5

The results obtained above from the three groups will be briefly summarized. In the case of *pH* 7.2, of 100 vol. % of total CO_2 gas content in blood, 93 vol. % are produced by carbonate and only 7 vol. % exist in free CO_2 gas state. When oysters are kept in fresh water the *pH* increases gradually during the period of experimentation. This increase is evidently due to the alteration in the component of the blood, or in this case the blood tends to enhance the quantity of fixed CO_2 gas which in turn diminishes the free CO_2 gas. After about two weeks in

fresh water the blood pH increases to 7.4. The rate of increase is not always proportional to the duration of immersion and often shows a distinct increase in the course of two weeks. Indeed, I have seen the fixed CO_2 increased to 95 vol. % and free CO_2 decreased to 5 vol. % of total CO_2 gas content in the blood.

In normal blood of the oysters, according to KOKUBO ('29), CO_2 gas content is about 4.22 vol. %. Since the water containing 4.22 vol. % of CO_2 gas in solution should freeze at $-0.007°C$ by calculation and hence the blood of oyster freezes at $-1.945°C$ (YAZAKI, '29) we find that the rôle of the osmotic concentration of the CO_2 is about $\frac{0.007}{1.945} \cdot .0.4/100 = 0.004$ of the total concentration and that of NaCl is about 93/100 (YAZAKI, '29). Therefore, NaCl and CO_2 produce $0.4 + 93 = 93.4$ per cent. of the total osmotic pressure in the normal condition. The remaining unaccounted 6.6 per cent. of the osmotic pressure must be influenced by some other causes.

Now,. the quantity of NaCl contained in the blood of dying oysters ($\Delta = 1.123$) is given in Table 4.

TABLE 4.

No. of experiment	No. of oysters used	Amount of NaCl (grs. per liter)	NaCl Δ' (calculated)	Δ'/Δ - 1.123
I	5	16.75	1.055	0.94
II	5	15.03	1.010	0.90
III	5	15.88	1.000	0.89
Mean		16.22	1.022	0.91

From the above, it may be said that 91 per cent. of the total osmotic pressure of the blood is exerted by NaCl.

While, on the contrary, CO_2 content in the blood of dying oysters, $\Delta = 1.123$, shows a marked increase as will be seen from the following table. (Table 5).

TABLE 5.

No. of experiment	No. of oysters used	Amount of CO_2 (vol. %)	CO_2 Δ' (calculated)	Δ'/Δ
I	5	4.5	0.008	0.007
II	5	5.4	0.009	0.008
III	5	5.8	0.010	0.009
Mean		5.2	0.009	0.008

It is seen from the table that the values of Δ'/Δ are about two times of the original values (0.004), that is, CO_2 gas influences 0.8/100 of the total osmotic pressure, therefore, NaCl and CO_2 produce $91+0.8=91.8$ per cent. of the total osmotic pressure in the above mentioned condition. From the results, the total osmotic pressure of the blood, in so far as it concerns with NaCl and CO_2 gas in dying period, in which the lowering of the blood concentration decreases very slowly, is maintained by NaCl and also by the rapid increase of the fixed CO_2 dissolved in blood.

3) *Transferring the oysters from fresh water to original sea water.*

There are a few reports concerning the change of blood concentration of marine animals immersed in fresh water as well as when those returned to the original sea water.

DAKIN ('08) experimented with the blood ($\Delta=0.57$) of a large eel, ***Anguilla vulgaris***, taken from fresh water and placed abruptly into sea water (sp. gr. 20.3). After 24 hours in sea water the blood appeared quite normal to those living in the sea, giving the value of $\Delta=0.745$.

DUVAL ('28) reported that the blood of fresh water molluscs is always denser than in the outer medium, and if placed in sea water of various concentrations, it becomes about isotonic with medium after some days.

I found that when the oysters which were previously kept in tap water ($\Delta=0.025$) or in pond water ($\Delta=0.028$) in the laboratory for 15 days, were replaced in original sea water in which they lived, the diluted concentration of the blood quickly recovered to its original concentration. This observation was repeatedly made. The Δ found for the blood of oysters which were kept in fresh water for 15 days gave approximately the value of 1.123.

In the following table the range of variations are tabulated.

TABLE 6.

Blood Δ, kept 15 days in fresh water	Number of oysters examined
1.150–1.140	3
1.130–1.120	48
1.110–1.100	9
Mean 1.123	

When such oysters were replaced in the original sea water, I can notice that, in the first period, the freezing point depression increases very rapidly in the first 5 hours or to about 84 per cent. of the original concentration at the rate of, from 0.105 to 0.07 an hour. In the next period, that is, after about five hours, the increase of the freezing point depression becomes slower showing the ratio 0.03 an hour and continues for about three hours showing the rise of about 91 per cent. of the normal concentration at the end of the time. In the third period, the increase of the freezing point depression becomes much slower and to reach approximately the original level, and it takes a considerably long period. The increasing ratio in the last instance is about from 0.011 to 0.006 an hour. The above relation is shown in detail in Table 7 and Fig. 3.

TABLE 7.

Time in hour	No. of oysters used	Blood Δ	$c=0.15\,t^{0.05}+1.123$	Increased Δ an hour	Relative increasing ratio an hour
0	6	1.123	1.123		
1	,,	1.241	1.273	0.118	0.105
2	,,	1.359	1.358	0.118	0.095
3	,,	1.458	1.429	0.099	0.073
4	,,	1.546	1.492	0.088	0.060
5	,,	1.655	1.550	0.119	0.070
6	,,	1.694	1.604	0.039	0.030
7	,,	1.733	1.654	0.039	0.024
8	,,	1.793	1.703	0.060	0.035
9	,,	1.812	1.749	0.019	0.011
10	,,	1.832	1.793	0.020	0.011
11	,,	1.850	1.836	0.018	0.010
12	,,	1.871	1.877	0.021	0.011
13	,,	1.889	1.899	0.018	0.010
14	,,	1.901	1.957	0.012	0.006
15	,,	1.912	1.995	0.011	0.006

From the above, it may be said that in the oysters which were previously immersed in fresh water and returned to sea water, the recovery took place within fifteen hours, while about 480 hours were needed in order to decrease the freezing point depression down to 1.123, indicating that the time required for recovery was less than 1/32 of the time required for depression. The difference between these two processes mentioned seems to indicate that it is to be attributed to some kind of a regulative capacity towards osmotic pressure posessed by the oyster.

The relation between the time required and the concentration altered may be mathematically expressed. The relation between c (the concentration and t (the time in hour) is expressed by an exponential formula:

$$c = A\,t^{x} + B$$

where A and B are the constant, and x the specific exponent.

From the actual data (Table 7) the numerical constants were calculated and given:

$$c = 0.15\,t^{0.65} + 1.123$$

The values found from the formula are shown in Table 7.

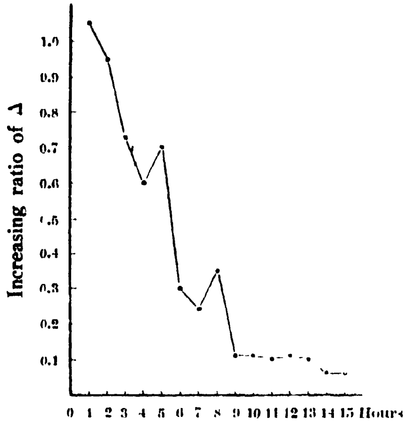

Fig. 3. Curve of the velocity of the increasing Δ. Showing are lation between the time elapsed and the ratio of relative increase of Δ.

SUMMARY.

1. Changes of blood Δ occur when the oysters are transferred from sea water to fresh water. The relation between the blood concentration (c) and time (t) can be expressed by the following equation:

$$t = 7.6 + Cot\,4.5°(c - 62).$$

When the oysters, which were previously kept in fresh water for several days and returned to normal sea water, the increasing Δ of the blood can be expressed by the equation:

$$c = 0.15\,t^{0.65} + 1.123$$

2. The oysters placed in fresh water may die sonner or later when the value of the blood Δ becomes from 1.20 to 1.00.

3. The blood of the oyster kept in fresh water shows increase in the fixed CO_2 and decreases in the free CO_2 in the course of several days.

4. The cause of the slow lowering of the osmotic concentration is maintained by NaCl and also by the rapid increasing of the fixed CO_2 dissolved in blood.

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On the Daily Progress of Carbon Assimilation in the Shadow under Natural Conditions.[1]

BY

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(With 7 Text-figures.)

(Received March 9, 1932)

INTRODUCTION.

Assimilation of carbon dioxide is a most significant function of green plants. As the assimilation is affected by external conditions, the analytical investigation of the carbon assimilation under natural conditions is an important one. The recent progress of the methods of experimental ecology, moreover, has proposed a promising field of study in assimilation. The profound study was reported by KOSTYTSCHEW and his co-workers (1926, 1928, 1930). They investigated the daily progress of the carbon assimilation of sun plants under natural conditions at many places and pointed out the interesting facts. Recently HARDER and his co-workers (1931) studied the assimilation under special conditions in a desert and gained also important results.

The different behaviors of sun and shade plants under various conditions are studied by many investigators. Generally, under natural conditions the shade plants are supposed to assimilate carbon dioxide in small amounts but our knowledge along this line is deficient, so that the writer intended to investigate the assimilation of shade plants as well as the shade leaves of sun plants on the subalpine region.

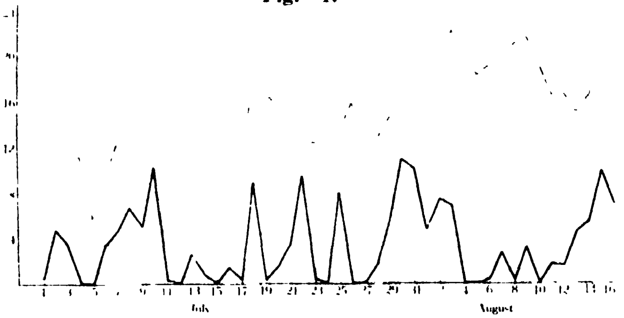
CLIMATIC CONDITIONS.

The snow in the botanical garden remained this year (1931) until the beginning of July, some ten days longer than the average year. The weather this summer was cloudy and cold, therefore, the temperature was very low and the humidity was comparatively high. The unfavorable conditions for the plant growth improved from the end of July, getting the temperature higher and lengthening the duration of sun shine. Even

[1] Contributions from the Mt. Hakkôda Botanical Laboratory. No. 11.

though bad weather prevailed at the beginning of August, the temperature for rainy weather was rather high. The unfavorable climatic conditions in the former half of July caused to some extent the delay of the flowering period.

Fig. 1.



——— Duration of sunshine in hours.
------- Temperature in °C.

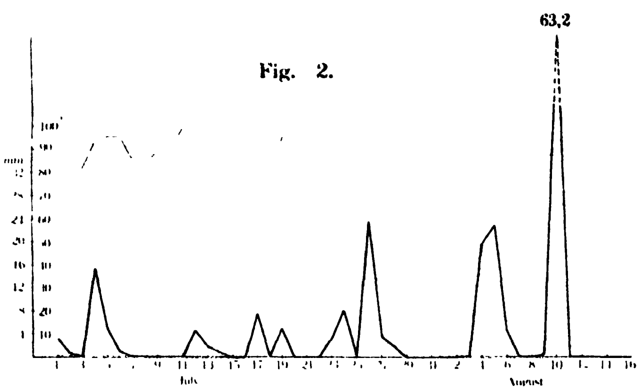


Fig. 2.

——— Rainfall in mm.
------- Relative humidity.

The climatic data during my stay at the Mt. Hakkôda Botanical Laboratory[1] as well as those of the foregoing period are shown in Fig. 1 and 2. The mean value of the temperature and relative humidity was obtained by four-hourly reading of the thermo-hygrograph. The duration of sun shine was taken from the JORDAN sun shine recorder.

[1] For the description of the situation of the laboratory and climate reference is made to a paper by YOSHII and JIMBO (1931).

METHOD AND MATERIALS.

Assimilation chamber used is of cubic glass, the free spaces beside the inserted leaves being left as small as possible. One opening, through which the leaves are inserted, is loosely closed with cotton in order to give the air free passage. The other small pore, situated against the former, is connected with the absorption tube. In the experiment the assimilation chamber is supported by a stand placed beside the plant to keep the leaves in their original position. Soon after each experiment, the leaves are removed from the chamber and left in the open air till the next experiment. The two plants grow near each other and so the leaves of the shade plant and the shade leaves of the sun plant were experimented upon at the same time, resulting in the leaves being found in the same direction side by side.

The duration of experiment is ca. 25–30 minutes. The experiments are carried out with the apparatus and the methode of BOYSEN-JENSEN (1928). In the experiment the two assimilation chambers, into which the leaves of two plants are inserted, are placed parallel to each other and connected with the absorption tubes and aspirators. For the control, the open air is suctioned through the glass tube, which is placed between the two assimilation chambers.

The amount of assimilation is reduced to the unit of 30 minutes and 50 sq. cm. surface area of the leaf.

The volume of air used is not so large as in the experiments of KOSTYTSCHEW (1928). As the result of repeated experiment with shade plants it was found that the volume of air needed for sufficient assimilation was only one-fourth liters per unit area of leaves per hour. 5 liters of air were used in every experiment, as the largest leaves of 40 sq. cm. were often used.

The temperature is obtained from a thermo-hygrograph and from the readings of thermometer which is placed near by, preventing from insolation.

The light intensity in the shade is measured by the wedge-photometer of EDER-HECHT. The photometer is exposed between the two assimilation chambers during the experiments.

EXPERIMENTS.

For the experiments shade plants and sun plants which grew in the alpine botanical garden of our institute were used. They are *Skimmia japonica*, *Streptopus ajanensis*, var. *japonica*, *Paris tetraphylla* and *Mitchella repens*, var. *undulata* (shade plants), and *Sasa kurilensis* (syn. *Pseudosasa*

kurilensis, MAKINO), *Acer Tschonoskii*, *Rhododendron brachycarpum* and *Ilex Sugeroki*, subsp. *brevipedunculata* (sun plants).

The results obtained are as follows:

1) *Skimmia japonica* and *Sasa kurilensis*.

Skimmia, one of the small shade shrubs on Mt. Hakkôda, are found under the *Sasa*-plants which grow luxuriantly near and around the botanical garden. The comparative experiments with *Skimmia* and *Sasa* were, therefore, carried out with much convenience. The east side of the place, where the experiments were done, was open and some leaves of those plants

TABLE 1.

July 22. *Skimmia japonica.* leaf area 16.3 sq. cm.
Sasa kurilensis. ,, ,, 29.7 ,, ,,

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm		Remarks	
Beginning	Duration in minutes										
	Skim.	*Sasa*				*Skim.*	*Sasa*	*Skim.*	*Sasa*	*Skim.*	*Sasa*
a.m. 5.00	24	25	fine, (before sunrise)	11.2°C.	9.18	0.08	-0.28	0.30	-0.55		
6.07	31	30	fine	12.2	18.94	0.30	0.28	0.88	0.46		
7.54	23	27	,,	14.3	45.8	0.25	0.08	1.01	0.15	Solar spots on 1/3 part of the leaf.	
8.57	36	33	,,	16.0	29.6	0.25	0.49	0.63	0.76	Solar sports on the leaf.	Solar spots on the leaf.
10.21	28.5	29.5	fine, later clouded.	17.2	214.1*	0.28	0.19	0.89	0.33	Direct light on almost all parts at the beginning and solar spots on the leaf in the later half of the experiment.	Direct light on almost all parts at the beginning and solar spots on the leaf in the later half of the experiment
11.37	29	26	fine	17.8	78.5	0.11	0.16	0.35	0.32	Solar spots on the leaf for 2′ or 3′.	
p.m. 1.34	26	28	,,	17.0	83.6	0.07	0.11	0.27	0.20		
2.56	28.5	29.5	cloudy	13.0	28.6	0.14	0.19	0.46	0.33		
4.11	26.5	30	changeable	12.0	37.4	0.22	0	0.76	0		
5.22	30	31	fine	11.0	11.90	-0.06	-0.03	-0.17	-0.05		

* Photometer was also insolated.

TABLE 2.

July 23. *Skimmia japonica.* leaf area 16.9 sq. cm.
Sasa kurilensis. „ „ 37.3 „ „

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.	
Beginning	Duration in minutes								
	Skim.	*Sasa*				*Skim*	*Sasa*	*Skim.*	*Sasa*
a.m. 5.03	27	29	cloudy	10.8 C.	7.02	0	-0.20	0	-0.28
6.29	29	29	„	11.3	14.63	0.06	0.07	0.17	0.10
8.21	23	26	slightly clouded	13.0	23.4	0.25	0.13	0.96	0.20
10.07	29	31	changeable	15.0	67.4	0	0.09	0	0.12
11.42	29	30	cloudy, rain for 5 minutes.	13.5	13.71	0.11	-0.15	0.34	-0.20
p.m. 1.53	30	29	rain	13.2	14.63	-0.03	0.06	-0.10	0.08
3.23	28	31	rainy cloudy	14.2	9.50	0.19	-0.20	0.60	-0.26
4.53	28	26	„	13.8	4.95	0.02	0	0.07	0
6.21	29	30	fine	13.0	3.34	-0.04	-0.08	-0.10	-0.11

TABLE 3.

August 12. *Skimmia japonica.* leaf area 27.5 sq. cm.
Sasa kurilensis. „ „ 34.7 „ „

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.	
Beginning	Duration in minutes								
	Skim.	*Sasa*				*Skim.*	*Sasa*	*Skim.*	*Sasa*
a.m. 6.08	26	29	cloudy	15.0°C.	6.02	0.31	0.09	0.66	0.13
7.40	25	23	„	15.9	8.94	0.13	0.09	0.30	0.16
8.43	27	27.5	slightly clouded	17.8	15.73	0.30	0.17	0.61	0.27
9.40	26	25.5	cloudy	18.0	12.91	0.16	0.09	0.35	0.14
10.40	27	28	„	19.5	18.58	0	0.02	0	0.03
11.41	23	24	„	19.1	21.7	0.05	0.06	0.14	0.11

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per $^1/_2$ hour, per 50 sq. cm.	
Beginning	Duration in minutes								
	Skim.	*Sasa*				*Skim.*	*Sasa*	*Skim.*	*Sasa*
p.m. 1.16	29.5	31	changeable	20.5	19.20	0.19	0.12	0.35	0.16
2.17	25	25	,,	19.9	18.80	-0.03	0	-0.06	0
3.28	28	27	cloudy	19.0	10.34	0.08	0.06	0.16	0.10
4.37	24	25	,,	17.8	5.16	0.05	0.02	0.12	0.03
5.46	28.5	30	,,	16.7	3.13	0	-0.11	0	-0.15

TABLE 4.

August 13. *Skimmia japonica.* leaf area 17.7 sq. cm.
Sasa kurilensis. ,, ,, 37.3 ,, ,,

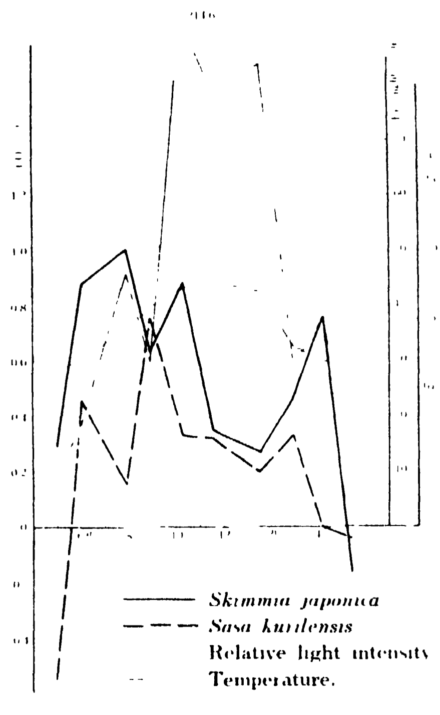
Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per $^1/_2$ hour, per 50 sq. cm.		Remarks	
Beginning	Duration in minutes										
	Skim.	*Sasa*				*Skim.*	*Sasa*	*Skim.*	*Sasa*	*Skim.*	*Sasa*
a.m. 4.42	27	26	fine, (before sunrise)	13.3°C.	3.24	-0.03	-0.16	-0.08	-0.25		
5.43	31	34	fine	14.7	4.13	0.08	0.03	0.22	0.03		
7.12	23	24	,,	17.8	11.88	0.14	0.22	0.50	0.37	Solar spots on the leaf.	Solar spots on the leaf.
8.13	29.5	30	slightly clouded	18.7	9.80	0.14	0.13	0.39	0.18		
9.12	25	23	changeable	20.2	14.40	-0.05	-0.11	-0.18	-0.19		
10.10	25	27	fine, cloudy	22.0	21.96	0.13	0.19	0.44	0.27		Solar spots on the leaf.
11.12	25.5	25.5	fine, cloudy	21.6	19.69	0	-0.02	0	-0.04	Solar spots on the leaf for 5 minutes at the beginning	,,
p.m. 12.46	29	30.5	,,	21.0	18.94	0.08	0.30	0.24	0.40		
1.49	25	24.5	,,	22.5	23.0	0.11	0.08	0.37	0.13	Direct light on $^1/_2$ part of the leaf for 7 minutes.	Direct light on $^1/_3$ part of the leaf.
2.48	26	28	cloudy	20.5	9.18	0.11	0.11	0.36	0.16		
3.47	26	24	,,	19.3	10.58	0.11	0.16	0.36	0.29		
4.50	29.5	27	,,	18.8	4.30	0	-0.05	0	-0.08		
5.52	26	26	,,	18.0	3.41	-0.04	-0.13	0.14	-0.20		

were often exposed to the direct sunlight in the morning, and even in the afternoon the solar spots fall on the leaf.

All the arrangements for the experiments were prepared on the previous day, without inserting the leaves into the assimilation chamber.

On the first day (July 22) the weather was fair but the temperature was very low, scarcely reaching 18°C. at midday. The plant leaves were insolated in the morning and solar spots shined on the leaves frequently. The assimilation of *Skimmia* increased very rapidly early in the morning with the increase of the light intensity (Table 1 and Fig. 3), but decreased afterwards. When insolated, however, it raised again (at 10:21 a.m.). Near midday a remarkable depression of assimilation occurred, even though strong light intensity and high temperature were observed. The rapid increase of assimilation later in the afternoon was again observed. The shape leaves of *Sasa* behaved similarly to the above described *Skimmia* excepting that the amount of assimilation was usually smaller and the increasing later in the afternoon was not observed.

Fig. 3.



The following day (July 23) was rainy with a lower temperature and weaker light intensity. Even under such conditions the assimilation of *Skimmia* was comparatively great (Table 2 and Fig. 4). The strongest assimilation was observed in the morning soon before the light and temperature reached their maxima. The assimilation was much depressed in the morning when the light intensity and the temperature were at the maximum. At noon a depression of the assimilation was not observed but it was observed before and after noon. It is worthy to note that the assimilation of *Sasa* was very small on this rainy day as compared with *Skimmia*, the similar result was also observed on August

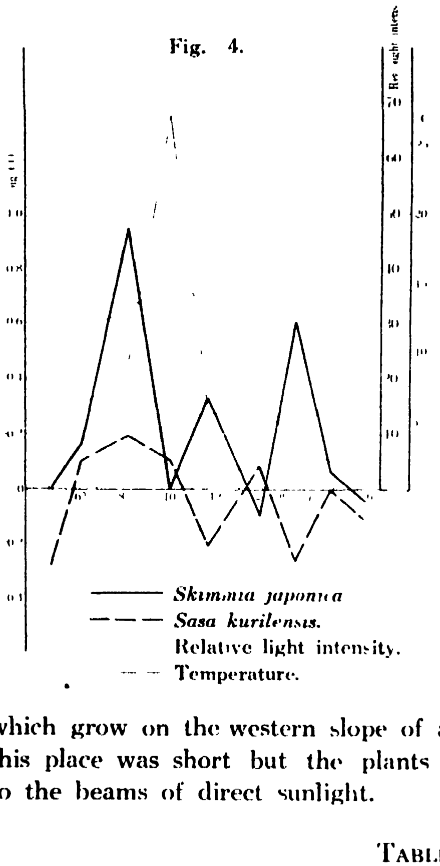


Fig. 4.

12, though the temperature was a little higher (Table 3). Moreover, it is peculiar that the curve of assimilation of *Sasa* did not run similarly to that of *Skimmia*, i. e. remarkable depressions were observed about noon and in the afternoon.

Another experiment, carried out on August 13, showed that the depressions of assimilation took place in the morning earlier than those appeared on the July days (Table 4). The curve of assimilation runs fairly parallel with that of the light when the depression of assimilation does not occur.

2) *Paris tetraphylla* and *Rhododendron brachycarpum*.

The experiments were carried out with the above two plants which grow on the western slope of a hill. The duration of insolation in this place was short but the plants were exposed in the afternoon near to the beams of direct sunlight.

TABLE 5.

July 25. *Paris tetraphylla.* leaf area 36.6 sq. cm.
Rhododendron brachycarpum. „ „ 38.8 „ „

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.		Remarks	
Beginning	Duration in minutes										
	Paris	*Rhod.*				*Paris*	*Rhod.*	*Paris*	*Rhod.*	*Paris*	*Rhod.*
a.m. 6.22	30	31	fine	13.1°C.	5.82	0.05	0	0.07	0		
7.29	32	32	changeable	14.0	12.00	0.20	0.20	0.25	0.23		
9.03	30	27	fine	15.0	20.53	0.24	0.21	0.32	0.31	Solar spots on the leaf.	Solar spots on the leaf.

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.		Remarks	
Beginning	Duration in minutes										
	Paris	*Rhod.*				*Paris*	*Rhod.*	*Paris*	*Rhod.*	*Paris*	*Rhod.*
10.39	27	26	fine	17.0	22.6	0.05	0	0.07	0	Direct light on the large part of the leaf.	Solar spots on the leaf.
11.47	29	30	fine, clouded for 6 minutes.	17.0	28.6	-0.11	0.10	-0.15	0.13		
p.m. 1.36	32	32	cloudy	17.5	25.6	-0.05	-0.24	0.07	-0.28		
3.17	32	31	,,	16.2	19.83	0.02	0.02	0.02	-0.03		
4.27	25	25	cloudy	14.0	8.03	0.27	0.03	0.40	0.01		
5.32	29	26	cloudy, later fine	13.8	5.36	0.24	0.20	0.30	0.30		
6.30	30	32	fine	13.0	2.24	0.08	-0.12	-0.11	-0.14		

TABLE 6.

July 26. *Paris tetraphylla.* leaf area 36.6 sq. cm.
Rhododendron brachycarpum. ,, ,, 38.0 ,, ,,

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.	
Beginning	Duration in minutes								
	Paris	*Rhod.*				*Paris*	*Rhod.*	*Paris*	*Rhod.*
a.m. 5.00	30	31	rainy	13.5°C.	2.92	0.07	-0.11	-0.10	0.15
6.33	29	26	,,	14.0	6.55	0.03	0	0.04	0
8.22	31	30	rainy, strong wind	14.3	7.33	0.05	0.02	0.07	0.02
10.12	30	27	,,	15.0	22.6	0.29	0.22	0.41	0.34
11.37	30	31	rainy	17.0	15.68	0.17	0.26	0.24	0.32
p.m. 1.32	26	25	rainy, strong wind	17.0	20.53	0.36	0.27	0.56	0.42
2.54	31	31	,,	17.0	9.80	-0.16	-0.12	-0.21	-0.13
4.25	27	27	rainy	14.7	4.37	0.05	-0.14	0.08	0.22
5.58	31	30	,,	14.5	4.09	0.07	-0.12	-0.10	-0.16

TABLE 7.

July 27. *Paris tetraphylla.* leaf area 22.8 sq. cm.
Rhododendron brachycarpum. „ „ 37.1 „ „

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.	
Beginning	Duration in minutes								
	Paris	*Rhod.*				*Paris*	*Rhod.*	*Paris*	*Rhod.*
a.m. 5.24	29	30	rainy cloudy	12.0°C.	3.82	0.05	0.23	-0.13	-0.31
6.49	29	30	„	12.2	8.02	0.03	0.11	0.06	0.17
8.25	30	32	cloudy	13.0	8.57	0.05	-0.05	0.12	-0.07
9.47	29	28	rainy cloudy	14.5	16.74	0.17	0.24	0.37	0.33
11.33	25	26	cloudy	17.0	26.8	0.03	-0.07	0.07	-0.11
p.m. 1.44	32	30	changeable	16.0	22.6	0.22	0.31	0.45	0.48
3.08	31	31	cloudy	16.5	15.68	0.22	0.21	0.47	0.30
4.44	31	30	„	14.3	5.55	0.03	-0.06	0.05	-0.09
6.11	28	27	„	13.8	4.28	-0.06	-0.17	-0.12	-0.26

TABLE 8.

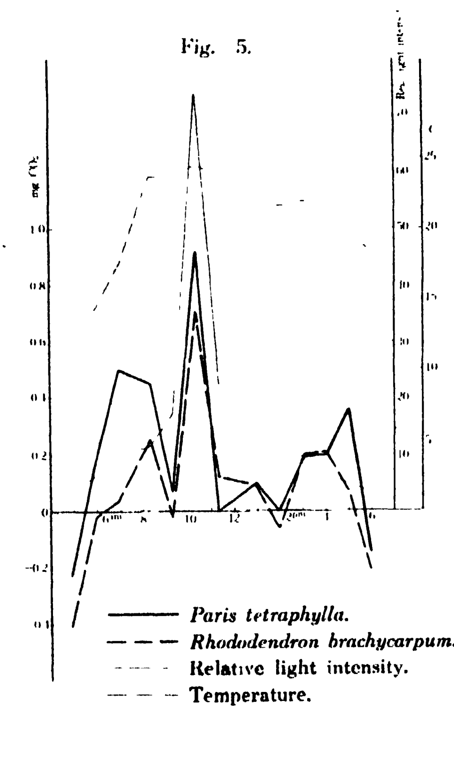
August 11. *Paris tetraphylla.* leaf area 31.5 sq. cm.
Rhododendron brachycarpum. „ „ 39.5 „ „

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.		Remarks	
Beginning	Duration in minutes										
	Paris	*Rhod.*				*Paris*	*Rhod.*	*Paris*	*Rhod.*	*Paris*	*Rhod.*
a.m. 4.53	23	30	fine, (before sunrise)	13.0°C.	2.64	-0.13	-0.33	-0.23	-0.41		
5.54	27	28	fine	14.3	6.23	0.08	0.03	0.17	-0.03	Solar spots on the leaf for 5 minutes.	Solar spots on the leaf for 2 minutes.
6.56	26.5	30	„	17.5	9.80	0.27	0.04	0.50	0.04		
8.20	27	25	„	23.7	11.56	0.24	0.15	0.45	0.26		
9.18	29	29	changeable	23.7	17.31	0.04	0.02	0.07	-0.02	Solar spots on the leaf frequently.	

Experiment: Beginning	Duration in minutes: Paris	Duration in minutes: Rhod.	Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2: Paris	Assimilation in mg. CO_2: Rhod.	Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.: Paris	Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.: Rhod.	Remarks: Paris	Remarks: Rhod.
10.19	24	24	changeable	24.0	73.8	0.47	0.45	0.92	0.71	Direct light on 1/3 part of the leaf.	Direct light on 1/3 part of the leaf.
11.19	25.5	24	slightly clouded	23.8	22.3	0	0.07	0	0.12		
p.m. 12.58	25	25	cloudy	22.5	11.78	0.06	0.07	0.10	0.09		
1.58	24	24.5	,,	21.6	12.48	0	-0.05	0	-0.06		
3.01	27	25	,,	21.9	9.18	0.11	0.11	0.19	0.20		
4.01	27	26	,,	20.7	6.46	0.11	0.14	0.19	0.21		
5.00	25.5	25	,,	19.6	4.82	0.18	0.05	0.36	0.07		
5.59	26	25.5	fine, later clouded	18.3	3.15	-0.11	0.14	-0.15	0.21		

On a very fine day (July 25) the assimilation depressed near noon time, presenting a bimaximal curve (Table 5). By the depression of assimilation even the output of carbon dioxide was distinctly observed. This is a remarkable phenomenon which was not observed in the experiment of *Skimmia* and *Sasa* on a fine day. On a rainy day (July 27) the same tendency was shown (Table 7) but the pause of assimilation was not as long as on July 25. In the experiment of the other fine day (August 14) the maximum of the curve was consistent with that of the light intensity (Table 8 and Fig. 5). The fall of the curve at noon moved to the early afternoon. In this experiment

Fig. 5.



the curve of assimilation is trimaximal.

Although the curve of assimilation was delayed (a rainy day, July 26) (Table 6), yet the curve of assimilation and light intensity run hand by hand, being quite similar as on August 14.

3) Streptopus ajanensis, var. *japonica* and *Acer Tschonoskii*.

These plants were found near the former plants, so that the light condition was practically the same as before.

The experiments were carried out on fine days. The results obtained in the morning of July 30 (Table 10) and in the afternoon of July 31 (Table 11 and Fig. 6) showed that the curves of assimilation run failry parallel with the light intensity. The progress of assimilation is trimaximal. In these experiments the increase of assimilation was observed in the

TABLE 9.

July 29. *Streptopus ajanensis*, var. *japonica*. leaf area 21.4 sq. cm.
Acer Tschonoskii. „ „ 36.8 „ „

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.		Remarks	
Beginning	Duration in minutes										
	Strept.	Acer				Strept	Acer	Strept	Acer	Strept.	Acer
a m. 5.23	29	31	cloudy	13.0°C.	4.37	0.14	0.11	0.33	0.14		
6.49	24	24	„	14.0	8.57	0.36	0.61	1.04	1.02		
8.20	29	30	cloudy, later fine	16.0	11.20	-0.14	-0.11	-0.23	-0.15		
9.45	26	25	fine	17.8	20.53	0	0.17	0	0.27		
11.36	30	30	„	19.0	32.7	0.03	0.08	0.06	0.11		Solar spots on the leaf frequently
p.m. 1.39	29	30	changeable	20.0	37.4	0.17	0.28	0.40	0.37	Solar spots on the leaves frequently	
3.08	30	30	cloudy	16.0	11.20	0.19	0.16	0.45	0.20		
4.38	29	32	„	16.0	7.34	0.06	0.03	0.13	-0.03		
6.10	32	32	slightly clouded	14.5	3.58	-0.06	-0.19	0.14	-0.25		

TABLE 10.

July 30. *Streptopus ajanensis*, var. *japonica*. leaf area 28.6 sq. cm.
Acer Tschonoskii. „ „ 38.8 „ „

Experiment: Beginning	Duration in minutes: Strept	Duration in minutes: Acer	Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2: Strept.	Assimilation in mg. CO_2: Acer	Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.: Strept	Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.: Acer	Remarks: Strept.	Remarks: Acer
a.m. 4.35	26	25	fine, (before sunrise)	10.5°C.	3.28	-0.03	-0.06	-0.05	-0.10		
5.26	29	29	fine	11.8	5.36	0.17	0.10	0.30	0.11		
6.31	28	27	„	13.6	8.02	0.17	0.18	0.31	0.25		
8.01	29	31	slightly clouded	16.8	12.80	0.22	0.13	0.40	0.16		
9.05	27	25	„	18.7	37.4	0.33	0.33	0.64	0.51	Direct light on half of the leaves.	Direct light on the 1/3 part of the leaf.
10.25	29	30	„	19.2	28.6	0.11	0.06	0.20	0.08		
11.44	26.5	27.5	„	19.8	17.34	0.25	0.45	0.49	0.64		Solar spots on the leaf.
p.m. 1.31	29	32	„	22.0	20.53	0.22	0.13	0.40	0.16	Weak solar spots on the leaves.	
3.00	27	28	„	20.2	20.53	0.03	0.02	0.05	0.03		
4.24	28	31	clouded, later fine	17.0	12 80	0.23	0.10	0.43	0.12		
5.25	28	25	cloudy	15.3	5.00	0	0.05	0	0.07		
6.22	29	31	fine	15.0	2.73	-0.11	-0.20	-0.20	-0.24		

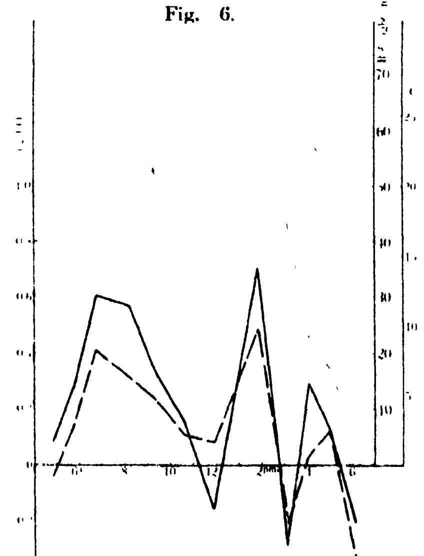
TABLE 11.

July 31. *Streptopus ajanensis*, var. *japonica*. leaf area 20.0 sq. cm.
Acer Tschonoskii. „ „ 38.3 „ „

Experiment: Beginning	Duration in minutes: Strept.	Duration in minutes: Acer	Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2: Strept.	Assimilation in mg. CO_2: Acer	Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.: Strept	Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.: Acer	Remarks: Strept.	Remarks: Acer
a.m. 4.50	25	24	fine, (before sunrise)	9.5°C.	4.44	0.03	-0.03	0.08	-0.04		
5.43	30	33	fine	12.0	7.02	0.11	0.11	0.27	0.13		
6.43	25	24	„	15.8	9.25	0.19	0.25	0.60	0.41		

Experiment: Beginning	Duration in minutes: Strept.	Duration in minutes: Acer	Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2: Strept	Assimilation in mg. CO_2: Acer	Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.: Strept	Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.: Acer	Remarks: Strept.	Remarks: Acer
8.10	29	28	fine	20.0	7.81	0.22	0.22	0.57	0.31		
9.20	25	23	,,	21.2	53.2	0.11	0.14	0.33	0.23	Direct light on almost all parts of the leaves.	Direct light on 1/3 part of the leaf.
10.37	28	30	fine later clouded	21.2	28.6	0.06	0.08	0.15	0.11		Solar spots on the leaf for 5 minutes.
11.57	25	26	cloudy	20.5	27.1	-0.05	0.06	-0.16	0.08		
p.m. 1.52	29	29	changeable	22.6	69.8	0.28	0.35	0.71	0.49	Direct light on all parts of the leaves, when sun shines.	Direct light on all parts of the leaf when sun shines
3.10	28	26	fine	25.0	42.7	-0.11	-0.14	-0.29	-0.21	Solar spots on the leaves for 5 minutes.	Solar spots on the leaf for 2 minutes.
4.06	28	32	,,	24.0	22.7	0.11	0.03	0.29	0.03	Week solar spots on the leaves for 8 minutes	Solar spots on the leaf for 5 minutes.
5.00	31	27	,,	20.5	17.34	0.05	0.09	0.13	0.12		
6.09	32	38	,,	16.0	4.12	-0.09	-0.32	-0.21	-0.33		

Fig. 6.



——— *Streptopus ajanensis*, var. *japonica*.
— — — *Acer Tschonoskii.*
– — Relative light intens'ty.
– – – Temperature.

late afternoon, but this did not hold for the shade leaves of *Acer*. In the experiment of July 31 the assimilation was depressed at noon and 3.10 p.m. and even the output of carbon dioxide was observed. This output of carbon dioxide occurred also on July 29 (Table 9).

On a cloudy day, (July 29) a strong assimilation was observed early in the morning, this phenomenon occurred only once throughout the experiments (Table 9).

4) ***Mitchella repens***, var. ***undulata*** and ***Ilex Sugeroki***, subsp. ***brevipedunculata.***

These plants grow on the eastern slope of a hill, thus being exposed to the direct sunlight in the morning. The leaves of these plants were very small, and for the sake of convenience the stems or branches were also inserted into the assimilation chamber. Since this shade plant (***Mitchella***) grows creepingly, in order to avoid the mixing of soil

TABLE 12.

August 2. *Mitchella repens*, var. *undulata*. leaf area 17.6 sq. cm.
Ilex Sugeroki, subsp. *brevipedunculata*. „ „ 24.6 „ „

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg CO_2, per $^1/_2$ hour, per 50 sq. cm.		Remarks	
Beginning	Duration in minutes										
	Mit.	*Ilex*				*Mit.*	*Ilex*	*Mit.*	*Ilex*	*Mit.*	*Ilex*
a.m 4.51	27	26	fine	16.5°C.	5.01	0.06	0	0.17	0		
5.50	30	29	fine, clouded for 2 minutes.	18.2	12.00	0.22	0.27	0.63	0.58		
6.50	25	25	changeable	21.2	22.7	0	0	0	0	Solar spots on the leaves, when it is fine.	Solar spots on the leaves, when it is fine.
8.12	27.5	28	cloudy, shine for 4 minutes.	21.3	20.3	0.14	0.09	0.43	0.18		
9.20	30	28	cloudy, shine for 3 minutes.	21.5	15.68	0.09	0.20	0.23	0.42		
10.26	28	32	cloudy	22.0	16.80	0.22	0.30	0.67	0.57		
11.31	26	29	cloudy, shine for 8 minutes.	23.3	22.6	-0.09	0.06	-0.27	0.12		
p.m. 1.05	32	32	changeable	25.3	15.3	0	-0.03	0	-0.05		
2.14	30	28	changeable	24.0	18.94	0.08	0.09	0.23	0.18		
4.00	33	34	cloudy, shine for 3 minutes.	21.7	8.08	-0.03	-0.03	-0.08	-0.05		
5.35	25.5	27.5	cloudy	20.5	5.47	-0.06	-0.06	-0.18	-0.12		

TABLE 13.

August 3. *Mitchella repens*, var. *undulata*. leaf area 17.6 sq. cm.
Ilex Sugeroki, subsp. *brevipedunculata*. „ „ 23.2 „ „

Experiment: Beginning	Duration in minutes: *Mit.*	Duration in minutes: *Ilex*	Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2: *Mit.*	Assimilation in mg. CO_2: *Ilex*	Assimilation in mg. CO_2, per $^1/_2$ hour, per 50 sq. cm.: *Mit.*	Assimilation in mg. CO_2, per $^1/_2$ hour, per 50 sq. cm.: *Ilex*
a.m. 4.38	29.5	32	fine	18.0°C.	2.86	-0.05	-0.14	0.15	-0.28
5.40	26	28	cloudy	18.5	5.36	0.03	-0.05	0.09	-0.12
6.47	34.5	35	changeable	21.7	10.28	0.27	0.25	0.68	0.46
8.14	23	23	„	23.3	12.39	0.19	0.11	0.71	0.31
9.38	31	31	cloudy, shine for 10 minutes.	23.4	16.79	0.08	0.09	0.23	0.17
10.57	26	26	cloudy	23.8	19.30	0.30	0.36	1.00	0.89
p.m. 12.47	32	32.5	„	25.3	15.70	0.08	0.11	0.22	0.22
1.56	29	28	changeable	25.3	10.77	0.13	0.22	0.40	0.51
3.15	32	34	cloudy, shine for 5 minutes.	22.8	8.62	0.01	0.08	0.03	0.16
4.37	26.5	27	changeable	21.2	7.29	0.30	0.19	0.97	0.46
6.03	29	28.5	fine	19.7	3.02	-0.05	-0.08	-0.16	0.19

TABLE 14.

August 4. *Mitchella repens*, var. *undulata*. leaf area 18.8 sq. cm.
Ilex Sugeroki, subsp. *brevipedunculata*. „ „ 23.2 „ „

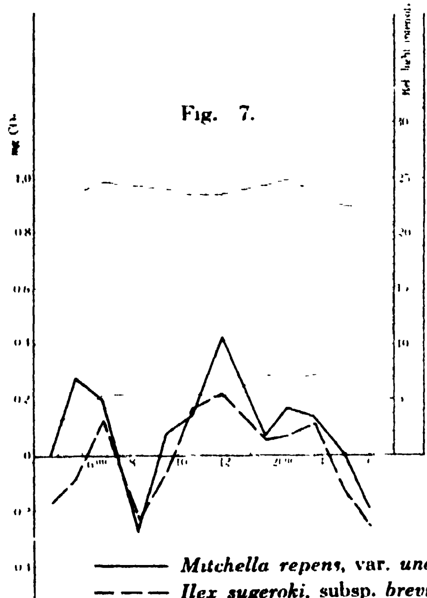
Experiment: Beginning	Duration in minutes: *Mit.*	Duration in minutes: *Ilex*	Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2: *Mit.*	Assimilation in mg. CO_2: *Ilex*	Assimilation in mg. CO_2, per $^1/_2$ hour, per 50 sq. cm.: *Mit.*	Assimilation in mg. CO_2, per $^1/_2$ hour, per 50 sq. cm.: *Ilex*
a.m. 4.39	30.5	30	cloudy, (before sunrise)	17.9°C.	2.73	0	-0.09	0	-0.18
5.42	31	33	rainy cloudy	18.9	3.91	0.11	-0.04	0.28	-0.08

Experiment			Weather	Temperature	Relative light intensity	Assimilation in mg. CO_2		Assimilation in mg. CO_2, per 1/2 hour, per 50 sq. cm.	
Beginning	Duration in minutes								
	Mit.	*Ilex*				*Mit.*	*Ilex*	*Mit.*	*Ilex*
6.53	26	28	cloudy	19.7	5.36	0.07	0.06	0.20	0.13
8.19	31	30	rainy cloudy	19.5	5.53	-0.11	-0.12	-0.28	-0.24
9.31	28	28.5	,,	19.2	7.02	0.03	-0.03	0.08	-0.06
10.35	30	31	,,	18.8	5.53	0.05	0.09	0.14	0.17
11.51	25	24	,,	19.0	5.16	0.14	0.09	0.43	0.22
p.m. 1.38	31	31.5	,,	19.5	7.23	0.03	0.03	0.07	0.05
2.30	25.5	26	,,	19.9	6.70	0.06	0.03	0.17	0.07
3.42	31.5	30.5	,,	19.0	6.98	0.06	0.06	0.14	0.12
4.58	28	27	,,	18.0	3.54	0	-0.06	0	0.13
6.01	33	34	,,	18.0	—	-0.08	-0.14	-0.20	-0.26

respiration, the ground under the assimilation chamber was covered with black paper, which did not reflect the light.

The curve of assimilation is trimaximal and does not run parallel with light and temperature. By the depression at noon (August 2) the shade plant showed an output of carbon dioxide (Table 12), but in the morning (August 4) the same was observed in both plants (Table 14 and Fig. 7). On these two days the assimilation ceased earlier in the afternoon than

Fig. 7.



———— *Mitchella repens*, var. *undulata*.
— — — *Ilex sugeroki*, subsp. *brevipedunculata*.
———·· Relative light intensity.
· — — Temperature.

usual. As shown by Table 12 the amount of assimilation in the morning was very large while that in the afternoon was very small. On a rainy day (August 4) the depression of assimilation at noon was not observed and moreover both the assimilation and the light intensity were very low (Table 14 and Fig. 7). On August 3 both plants assimilated intensely even in the late afternoon, especially *Mitchella* did abnormally (Table 13), and the maximum of assimilation was consistent with that of light, but not with that of temperature.

In these experiments the shade leaves of *Ilex* usually assimilated less than *Mitchella* throughout the experiments.

GENERAL DISCUSSION.

From the year 1926 to 1930, KOSTYTSCHEW and his co-workers investigated the daily progress of assimilation of many sun plants at various places under natural condition and observed the remarkable fluctuations of the assimilation and, moreover, they pointed out that the greater part of assimilation occurred in the morning.

The results of my experiments agree with them, with exception that the shade plants assimilate quite distinctly even in the afternoon. Although the output of carbon dioxide after the intense assimilation was observed as in the case of KOSTYTSCHEW, yet not so distinct. The output of carbon dioxide seems to have imporant significance for the shade plants, because the amount of assimilation by this plants is small. However, the shade plants assimilate quite large in the late afternoon but this did not always occur with the shade leaves of sun plants.

As McLEAN (1920) observed by coco-nut leaves, the depression of assimilation in my experiments occurs generally near noon time, the strongest intensity of light in the daytime.

The curves of assimilation are not consistent with that of light as a whole, but agree with it in parts. This may be attributed to the other factors which play also important parts by the assimilation.

Although the rising of temperature was observed on August days, compared with the July days, no distinct effect of temperature change on the assimilation was found by the experiments. These results may be explained by the characteristic assimilation of the shade plants, which usually assimilate very sparely under the various conditions.

Shade leaves of sun plants always assimilate less than the shade plants as shown by the tables. This is a distinct and interesting phenomenon from the ecological view point and is worthy of further study.

SUMMARY.

1. The experiments on the daily progress of assimilation of shade plants and shade leaves of sun plants under natural conditions were carried out in the Mt. Hakkôda botanical garden of our institute.

2. The assimilation in the morning is always stronger than that in the afternoon. Strong assimilation in the late afternoon is observed by the shade plant, but this was not always so in the case of shade leaves of sun plants.

3. A remarkable depression of assimilation usually occurs near noon time, excepting on rainy days. The curve of assimilation does not always run parallel with that of the light, especially no twith that of the temperature. It runs, however, in a similar manner on fine, rainy and cloudy days.

4. The shade leaves of sun plants usually assimilate less than those of the shade plants.

The writer wishes to express his hearty thanks to Prof. Dr. Y. YOSHII, under whose direction these experiments were carried out.

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Studies on the Exchange and the Equilibrium of Water and Electrolytes in a Holothurian, *Caudina chilensis* (J. MÜLLER)[1].

1. Permeability of the Animal Surface to Water and Ions in the Sea Water, together with Osmotic and Ionic Equilibrium between the Body Fluid of the Animal and its Surrounding Sea Water, involving some Corrections to our Previous Paper (1926).

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(With 13 text-figures.)

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CONTENTS.

[1] Contributions from the Marine Biological Station, Asamushi, Aomori-Ken. No. 85.

I. INTRODUCTION.

It has long been known that the marine invertebrates and elasmobranchs are readily permeable to water and, furthermore, the osmotic pressure of their blood and body fluids is almost similar to that of the surrounding medium. If the osmotic pressure of the medium is changed by dilution or concentration, their blood and body fluids are shown to change in the same direction and attain in a comparatively short time almost the same osmotic pressure, or their volume and weight increase or decrease correspondingly, showing the diffusion of water from the place of higher to one of lower osmotic pressure (BOTTAZZI, 1897, 1908; L. FREDERICQ, 1882, 1901; QUINTON 1897, 1900; DAKIN 1908; and many others). There are, of course, a few marine forms of invertebrates which can regulate the osmotic pressure of their body fluid to one different from that of the medium (see R. MARGARIA 1931).

Although it was considered by the majority of the investigators that the animal body is not permeable to salts (BOTTAZZI and P. ENRIQUES, 1901; V. HENRI and S. LALOU, 1903; L. FREDERICQ, 1904; A. B. MACALLUM 1903, 1904), the more recent researches by A. BETHE (1928, 1930) have shown both chemically and osmotically that the surfaces of the same species of animals employed by FREDERICQ and by BOTTAZZI (*Aplysia*, *Carcinus*) are not only permeable to water but also to the very salts in solution in the sea water and other substances. And the rate of diffusion of salts is, in general, slower than that of water, but is fast enough to be of physiological significance. The skin of these animals serves only as a protecting barrier to prevent the loss of the body colloids.

PANTIN (1931) also has described the electrolyte exchange of the estuarine flatworm, *Gunda ulvae*.

Most marine invertebrates, I think, are permeable to salts in the sea water; the question is, how freely are they permeable to each ion.

The present investigation is concerned with the way in which the surface of *Caudina chilensis*, including or excluding the mouth-alimentary-tract and anus-respiratory-trees, is permeable to water and to the predominating ions in the sea water.

The actual experiments were performed at the Asamushi Marine Biological Station but the chemical analyses were made at the Biological Institute at Sendai, from August 1929 to November 1931.

In this place I wish to express my sincere thanks to Prof. Dr. S. HATAI, Director of our Institute and of the Asamushi Marine Biological Station, for his kindness in giving me valuable criticisms during the course of my study and in reading this paper in spite of his precious time. I am also indebted to Assist. Prof. Dr. S. KOKUBO, Mr. E. SAWANO, Mr. T. TAMURA and all other members of the Station for their assistance and warm friendship during my stay at Asamushi.

II. MATERIAL.

The animals were collected from the shallow beach of Moura Bay, near the Asamushi Marine Biological Station, where they were found abundantly embedded in sand. The collected animals were kept in an aquarium with sand under the running sea water at the Station.

The healthy animals dig the sand and remain there as long as the conditions are satisfactory. The unhealthy ones are unable to burrow sand; they crawl out. All the animals used in this experiment were as fresh and as healthy as possible.

Next I shall describe some features of the animals necessary for the present investigation. (see N. KAWAMOTO 1927).

The animal is spindle-shaped, posterior part or tail, being elongated and slender. The body wall which consists of epithelium, connective tissue and circular muscles, is soft and translucent and on the inner surface of it five pairs of longitudinal muscles are attached in position which corresponds to the ambulacral zones. The tube feet are absolutely wating. The mouth, surrounded by tentacles, opens at the anterior end and the anus at the end of the tail. The genital papilla is situated near the mouth along the dorsal interradius. According to N. KAWAMOTO (1927), the body cavity is continuous with the exterior by the five pores in the anus which he called the "Cocloanal canals." To this point I will refer soon.

Most of the respiration of *Caudina* takes place in the respiratory trees through the cloacal chamber. Several c.c. of sea water are inhaled little by little until they entirely fill the respiratory trees, then exhaled at a breath while the sand which was swallowed through the mouth is thrown from the anus with the exhaled water (OGAWA 1927). This is regularly repeated at the temperature over 10°C. Since under 10°C. the respiration is irregular and slight, so I made experiments at the temperature over 15°C.

The body cavity is filled with a red colored fluid in which the alimentary tract, respiratory trees, gonad, *etc.* are bathed. The body fluid contains both red and white corpuscles to which its color is due. The circulation of the fluid was observed by Y. YAZAKI (1930). The body fluid normally does not coagulate in the air. In a certain case it is found divided into two phases, *i. e.*, agglutinated corpuscles and turbid liquid. If the body fluid is allowed to stand in a vessel, the corpuscles are deposited in a comparatively short time. The supernatant liquid is colorless or yellowish, always turbid in some measure with a small quantity of organic substances, such as protein.

III. THE COLLECTION AND THE TREATMENT OF THE BODY FLUID.

To obtain the body fluid, *Caudina* was removed from the aquarium, washed with water and again with distilled water, then wiped with a dry towel; the abdominal part of the body wall was cut carefully with scissors, so that the body fluid might not be contaminated by fluids or substances from the other organs, such as alimentary tract, gonad *etc.*, and the fluid was received in a hard glass beaker. About 5 to 20 c.c. of the body fluid were obtained from a single individual. The body fluid was then placed in a hard glass centrifuge tube with a cap and centrifuged for 3 to 5 minutes at 2000 to 3000 revolutions per minute so as to precipitate the blood corpuscles. The supernatant fluid was used for the present investigation.

IV. OSMOTIC AND IONIC EQUILIBRIUM BETWEEN THE BODY FLUID OF THE ANIMAL AND ITS SURROUNDING MEDIUM (NATURAL SEA WATER).

According to K. OKAZAKI and T. KOIZUMI (1926), the body fluid of *Caudina chilensis* is in osmotic equilibrium with sea water in nature and the predominating ions in the body fluid are very nearly in the same concentrations as those in which they occur in the sea water except that

Mg-ion in the body fluid being in a little lower concentration than in the sea water. Having doubts regarding this point, I have made the analyses again as much as it concerns with the question of the permeability of the animal surface to water and ions in solution in the sea water.

A. *The Specific Gravity, the Freezing Point Depression and the Electric Conductivity.*

1. Method.

Animals were kept in contact with the sea water in a large glass jar in the thermostat at least five hours to establish equilibrium, and were then used in the experiment. (see method of V.)

The specific gravity (d) was determined by means of the OSTWALD-SPRENGEL pyknometer. The freezing point depression (Δ) was determined by the BECKMANN thermometer, about 4 c.c. of the fluid being used. The specific conductance (κ) was measured by the WHEATSTONE bridge method, about 3 c.c. of the fluid being used.

All the determinations were carried out at constant temperature; the range of variation of the thermostat was $\pm 0.04°C$.

2. Experimental result.

The experiments are summarized in Tables I, II and III. They show

TABLE I.

Experiment No.	Specific gravity: $d_{19}^{18.53}$	
	Body fluid	Sea water
I	1.0250	1.0241
II	1.0248	
III	1.0252	

TABLE II.

Experiment No.	Freezing point depression: Δ	
	Body fluid	Sea water
IV	1.90	1.91
V	1.92	
VI	1.76	1.78
VII	1.77	
VIII	1.79	

TABLE III.

Experiment No.	Specific conductance: κ 25°±0.02	
	Body fluid	Sea water
IX	0.0486	0.0481
X	0.0481	0.0481

that the specific gravity, the freezing point depression, and the conductivity of the body fluid of *Caudina* are very similar to those of the surrounding sea water.

B. The Chemical Composition (Inorganic Analysis).

1. Method.

5 to 10 c.c. of the body fluid, freed from corpuscles, were measured accurately with an ordinary certified full-pipette (owing to its small viscosity) into a 50 c.c. measuring flask and 10 c.c. of distilled water and one drop of capryllic alcohol were mixed with the fluid. Then 12 to 13 c.c. of 12% solution of trichloracetic acid were added gradually, the flask being shaken gently while the acid is being added. After 10 minutes the content was made up to the mark by the addition of distilled water, and was well shaken and then filtered through a dry filter which is free from salts, while the first few c.c. of the filtrate were thrown away. 35 c.c. to 40 c.c. of the filtrate were accurately transferred into a hard glass ERLENMEYER flask by a full-pipette and the whole was evaporated to dryness under 100°C. When cooled in the desiccator the residue was dissolved in 30 to 50 c.c. of $\frac{1}{10}$ N. HCl solution measured accurately by means of a certified pipette. The resulting solution was stocked for the determinations of Na, K, Ca, Mg, and SO_4 ions. The determination of chlorine ion was performed, another sample being used.

The mode of the analytical procedure adopted, was, passing from ion to ion, to determine one of these in the whole series of samples; then similarly the second, *etc.* Each determination for a given sample was made at least twice, often three or five times, and the value ultimately adopted was the mean of two or three coinciding values.

No microanalysis is freed from errors; hence I did not always adopt the newest method of determination but adhered to one which was well practised by me; in each case a solution containing a known nearly identical amount of KAHLBAUM's guaranteed reagents was analysed simultaneously.

This operation was especially necessary to determine the potassium and calcium ions.

All the chemicals used in this investigation were "KAHLBAUM for analysis" or its substitute, and the vessels used for analyses ones of "Pyrex" or its substitute.

The sodium was determined by the KRAMMER-GITTLEMAN's method (1924), 2 c.c. of the sample being used for each determination, which correspond to ca. 1/5 volume of the body fluid; potassium by KRAMMER-TISDALL's method (1921), 2 c.c. of the sample being used and $NaNO_2$ added prior to precipitation to neutralize HCl; calcium by means of the CLARK-COLLIP's modification of the KRAMMER-TISDALL's method (1925), 2 c.c. of the sample being used and CH_3COONa being added before precipitation to displace HCl by CH_3COOH; magnesium on the calcium-free filtrate by the method of DENIS (1922), 2 c.c. being used; and the sulphate ion by E. G. WAKEFIELD's method (1929), 1 to 2 c.c. of sample being used except that I did not break up the precipitate in washing with acetone for fear of being uncapable of reprecipitation when being recentrifuged and that I did not adopt the colorimetry but adopted the acidimetry after FISKE, C. H. (1921). The source of errors of this acidimetric benzidine method for the determination of sulphur has been discussed by FISKE (1921) and by WILLIAM C. STADIE and EFFLE C. ROSS (1925). According to STADIE the quantitative precipitation of benzidine sulphate is independent of pH over a wide range from 7.0 to 1.6, and the ratio of benzidine hydrochloride to base in milliequivalents, may be varied from 1.4 to 4.0 with results within the limit of error (1%). These conditions were also satisfied in the present samples. The determination of chlorine ion was performed by the method of WHITEHORN (1921). Some determinations were made by the common method of VORHARD (in the case of studying the kinetics of osmotic equilibration).

2. Experimental results and discussion.

The experimental results are summarized in Table IV. They show that the inorganic composition of the body fluid freed from corpuscles, so far as $Na^{\cdot}$, $K^{\cdot}$, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, Cl', and SO_4'' are concerned, is very similar to that of the sea water which is in equilibrium with the animal.

As was stated above (II), we have 3 probable ways of permeability, the mouth-alimentary-tract, anus-respiratory-trees, and the body surface, and one way of communication, *i.e.* coeloanal canal after KAWAMOTO (1927). As to the first three ways, we may think of the influence of the colloid, namely protein in the body fluid. However, its concentration being

TABLE IV. — Inorganic Compositions of the Sea Water and the Body Fluid of *Caudina* in Equilibrium.

Experiment	Concentration in grs. per 100 c.c.						Dates of collection and of analysis.
	Cl′	SO_4''	Na·	K·	Ca··	Mg··	
Equilibrium sea water	1.910	—	—	—	—	—	Collection: Oct. 7, 1929. Analysis: Jan. 1930.
Animal No. 11	1.914	—	—	—	—	—	
12	1.916	—	—	—	—	—	
13	1.906	—	—	—	—	—	
14	1.906	—	—	—	—	—	
16*	1.907	—	—	—	—	—	
Equilibrium sea water	1.928	0.277	1.07	0.040	0.0425	0.123	Collection: Oct. 5, 1929. Analysis: from Feb. to Apr. 1930.
Animal No. 1′	—	—	1.02	0.043	0.0413	—	
3′	—	0.296	1.07	0.043	0.041	0.122	
4′	—	0.279	1.02	0.044	0.0419	0.130	
5′	—	0.296	1.06	0.040	0.043	—	
Equilibrium sea water	1.918	0.278	1.05	0.040	0.042	0.124	Collection: Oct. 6, 1929. Analysis: from Feb. to Apr. 1930.
Animal No. 7	—	0.283	—	—	0.0433	0.121	
8	—	0.276	1.04	0.044	0.045	0.123	
9	—	0.279	1.02	0.043	0.044	—	
Equilibrium sea water	1.879	0.260	1.00	0.041	0.039	0.132	Collection: Dec. 2, 1930. Analysis: from Dec. 2, 1930 to Jun. 1931.
Animal No. 10*	1.876	0.266	1.01	0.044	0.039	0.131	
11*	1.876	0.266	1.01	0.044	0.039	0.126	

16*: mixture from 8 animals.
10*: mixture from 4 animals.
11*: mixture from 2 animals.

very low and the microanalyses being not so exact, the DONNAN's membrane equilibrium (DONNAN, 1911; DONNAN and HARRIS, 1911; DONNAN and ALLMAND, 1914; DONNAN and GARNER, 1919), is out of question.

KAWAMOTO emphasized that he found 5 pores or coelo-anal canals at the posterior end of *Caudina*. Indeed we have occasionally observed the discharge of red colored body fluid from these 5 pores around the anus under varied circumstances such as on forcibly pulling the animal out of the sand, when it makes an effort in sand burrowing, or on being put in sea water without sand to burrow, or in unfavorable solutions, *viz.* hypertonic and hypotonic sea water, isotonic solutions of single salts such as KCl, $MgSO_4$ though not always the case with several other salts so far

tried. Since the animal which had contracted to small size while discharging the body fluid, in so far as I observed, was never restored to its original size or weight again, it seems to me that the inhalation of sea water from the pores is not the custom with *Caudina*.

Since, as regards the composition of corpuscles, I have only one sample of analysis, I shall reserve the data for some future occasion when I shall have made another analysis, and now content myself with describing that their ionic ratio is entirely different from that of the body fluid freed from corpuscles, owing to the presence of haemoglobin *etc.* as we see in blood of other animals; the concentration of inorganic phosphates, both in the body fluid (free from corpuscle) and in the corpuscles is as negligible as that in the sea water.

V. THE KINETICS OF OSMOTIC EQUILIBRATION AND IONIC PERMEATION.

The following study is concerned with the way in which the osmotic change measured by changes of the chlorine ion concentration, the freezing point depression, and the electric conductivity occurs while the water and ions diffuse through the animal surface including or excluding the mouth and anus, and with the rate of penetration of each ion in sea water through it.

A. *Theory.*

In order to account for the experimental fact of the permeability of the body wall of the animal to water and ions, quantitatively in terms of the osmotic laws, it is necessary to make certain assumptions concerning the animal and its surrounding medium:

1. The resistance to the diffusion of water and ions, per unit area of the skin, is constant.

This assumption was adopted by B. LUCKÉ, H. K. HARTLINE, and M. MCCUTCHEON (1931) in the case of the *Arbacia* egg. NORTHROP (1928), however, has considered the change of the membrane thickness due to the volume change produced by transport of the water. They agree with each other on neglecting the elasticity of the membrane on deriving the equation. This will be discussed later on.

2. The activity coefficients of the electrolytes are considered equal to unity for the sake of simplicity.

3. The concentration of the inner fluid and the surroundings are kept uniform. The time, required to equalize concentration differences within the body of the animal, across the alimentary tract between the body

fluid and the solution in the alimentary canal, is neglected as compared with that required for the water and (ions) to diffuse across the body wall.

This assumption makes the mathematical treatment of the permeability of body wall simple. This will soon be discussed.

4. The volume of the surrounding medium is so large that the concentration of each ion is unchanged in spite of exchanges of water and ions between the medium and the body fluid.

If we denote by —

C = the concentration of the osmotically active substances per unit volume of the inner fluid of the animal,

V = the volume of the inner fluid,

g = the total amount of the osmotically active substances of the inner fluid,

S = the permeability surface of the body wall of the animal,

C_m = the concentration of the osmotically active substances per unit volume of the medium,

t = the time,

then, we get —

$$C=\frac{g}{V} \quad \cdots\cdots\cdots\cdots \quad (1)$$

And differenciating (1) with respect to t,

$$-\frac{dC}{dt}=-\frac{\partial C}{\partial V}\frac{dV}{dt}-\frac{\partial C}{\partial g}\frac{dg}{dt}$$

$$=\frac{g}{V^2}\frac{dV}{dt}-\frac{1}{V}\frac{dg}{dt}$$

$$=\frac{C}{V}\frac{dV}{dt}-\frac{1}{V}\frac{dg}{dt} \quad \cdots\cdots\cdots\cdots \quad (2)$$

Here $\frac{dC}{dt}$ is the rate of change of concentration of the inner fluid. When the animal is not in osmotic equilibrium with the external medium, we observe the process of swelling or of shrinking.

Accordmg to the assumption 1 (and 3) the rate of transport of water across a unit area of the body wall depends only on the difference in the osmotic pressure (consequently the concentration) of the inside and outside solutions, and is appropriately assumed to be proportional to it as shown by other workers (LILLIE 1916; NORTHROP 1927, 1928; B. LUCKÉ, N. K. HARTLINE, and M. McCUTCHEON 1931 *etc.*).

This rate of transport of water is measured by the rate of change in the volume of the inner fluid or,

$$\frac{dV}{dt}=k'_1S(C-C_m)-k_1''S(C-C_m)=k_1S(C-C_m) \cdots\cdots \cdots (3)$$

in which k_1', k_1'' are the factors of proportionality and $-k_1''S(C-C_m)$ is the term due to the leakage ion.

And the force of diffusion of ions across the membrane is assumed to be proportional to the concentration gradient less (or more) the resistance produced by diffusion of water; or

$$-\frac{dg}{dt}=k_2'S(C-C_m)-k_2S''(C-C_m)=k_2S(C-C_m) \cdots\cdots\cdots (4)$$

where k'_2, k_2'' are the factors of proportionality.

In general, $$-\frac{dg_i}{dt}=k_{2i}S(C_i-C_{mi})\pm k_2''S(C-C_m) \cdots\cdots\cdots (4')$$

where i means i ion and C, C_m are the total concentrations of all kinds of ions. Substituting these value of $\frac{dg}{dt}$ and $\frac{dV}{dt}$ in (2), we obtain

$$-\frac{dC}{dt}=\frac{C}{V}\,k_1S(C-C_m)+\frac{1}{V}\,k_2S(C-C_m)$$

$$=\frac{S}{V}\,(C-C_m)(k_1C+k_2) \cdots\cdots\cdots\cdots\cdots\cdots\cdots (5)$$

On integration, assuming that $\frac{S}{V}$ is constant, we obtain:

$$0.4343\ K=\frac{1}{t(C_m+\frac{k_2}{k_1})}\log\frac{(C_0-C_m)}{(C-C_m)}\cdot\frac{(C+\frac{k_2}{k_1})}{(C_0+\frac{k_2}{k_1})} \cdots\cdots (6)$$

Here, log is the common logarithm; K is a constant and equal to $\frac{S}{V}k_1$; C_0 is the initial concentration of the inner fluid; C is the concentration of the body fluid at the time t; t is the time from the beginning of the experiment.

If as a first approximation $\frac{(C+\frac{k_2}{k_1})}{(C_0+\frac{k_2}{k_1})}$ is equal to 1, and $C_m+\frac{k_2}{k_1}$ is practically constant within the limit of change of C_m, the Equation (6) becomes a monomolecular one, or

$$0.4343\ K'=\frac{1}{t}\log\frac{C_0-C_m}{C-C_m} \cdots\cdots\cdots\cdots\cdots\cdots\cdots (7)$$

in which K' is $\frac{S}{V}(C_mk_1+k_2)$ and all other terms have the same meanings as before.

If total concentration of ions in both sides of the membrane is constant while concentration of each ion is different, it seems to me appropriate to assume $\frac{dV}{dt}=0$ in the Equations (2) and (3). Consequently the equation (4′) becomes

$$-\frac{dg_i}{dt}=k_{2i}'S(C_i-C_{mi}) \cdots\cdots\cdots (8)$$

On combining the Equations (8) and (2) one obtains —

$$-\frac{dC_i}{dt}=\frac{S}{V}k_{2i}'(C_i-C_{mi}) \cdots\cdots\cdots (10)$$

If one assumes that $\frac{S}{V}$ = constant, one obtains, on integration, a monomolecular equation, or

$$0.4343\ K''=\frac{1}{t}\log\frac{C_{oi}-C_{mi}}{C_i-C_{mi}} \cdots\cdots\cdots (9)$$

Here, log is the common logarithm C_{oi} is the initial concentration of i ion of the body fluid; C_{mi} is the concentration of i ion of the medium; C_i is the concentration of i ion of the inner fluid at the time t; t is the time from the beginning; K'' is the constant and equal to $\frac{S}{V}k_{2i}'$.

From Equations (3) and (4) we obtain —

$$\frac{\frac{dg}{dt}}{\frac{dV}{dt}}=\frac{dg}{dV}=\frac{\Delta g}{\Delta V}=\frac{k_2}{k_1} \cdots\cdots\cdots (10)$$

B. Method.

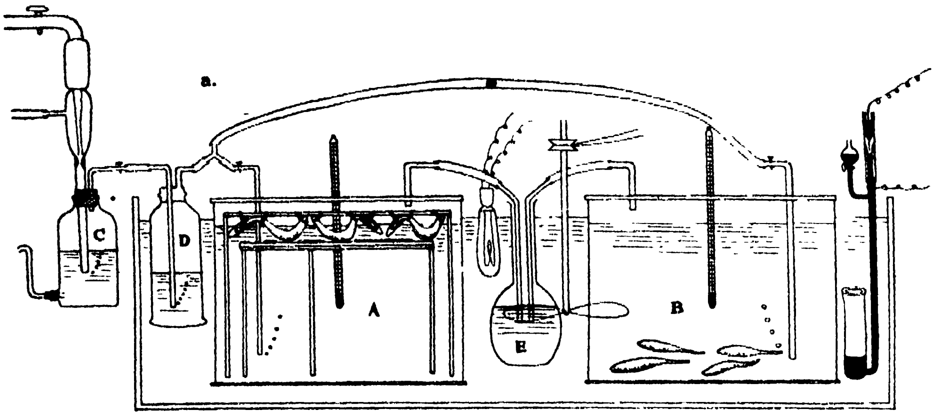
Given in Figs. I and II are diagrams of the apparatuses used.

In order to maintain uniform concentrations and temperature throughout the experiemental baths ((A), (A′), (B)) and also to keep the pH of the baths steady, and to supply oxygen, fresh outdoor air was passed through the baths during the course of the experiment by means of a water-blower which was fitted up as shown in (C). It consists of an aspirator and a bottle. Water, carrying air with it, passes into the bottle, where the water collects while the air passes out through the outlet tube into the baths.

The solution in (D) and in (E) is the same solution as is contained in the animal bath.

(A) is a 13 L. glass jar with a flat air tight ground-on lid. The lid is furnished with 3 holes, one for the thermometer, the others for inlet and outlet tubes. For supporting the animals, two frames of glass rods

a.



b.

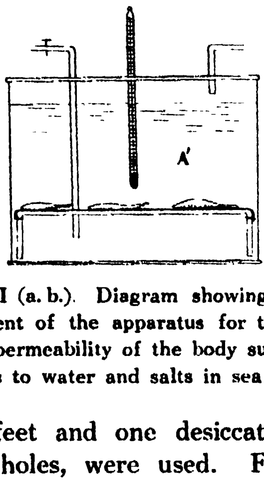


Fig. I (a. b.). Diagram showing the arrangement of the apparatus for the study of the permeability of the body surface of *Caudina* to water and salts in sea water.

Fig. II. Illustration of binding *Caudina* for the study of permeability of the body surface of *Caudina*, its mouth and anus being outside the experimental solution.

with feet and one desiccator plate, of glanzed porcelain, with several large holes, were used. For studying the permeability of animal body wall alone, after all possible exhalation of water from the respiratory trees takes place, their tails were put in the centrifuge tubes which are then bound to the animals in order to receive the water and inner fluid which may come from the anus, the animal was so tied to the glass frame with cotton strings that their mouths and anal parts together with centrifuge tubes were outside the solution as shown in Fig. II. (A') is similar to (A) except that for supporting animals, the glass-frame with short feet, and covered with a cotton gauze perforated with numerous holes is used instead of one porcelain plate and two glass frames. This was used for studying the permeability of the whole surface of the animal, including mouth and anus.

(B) is a similar jar to A and A′.

The whole apparatus except (C) was placed in the thermostat. The scullery of the laboratory at the Station served for the thermostat bath in which water was filled. For the heating element two electic carbon lamps, one for constant heating, the other for controlled heating, were employed. The heating current was controlled by means of an electromagnetic relay, in conjunction with a toluene-mercury thermo-regulator. The electric current employed both for heating and controlling was the alternating current of 100 V. The range of variation of the thermostat was ±0.04°C.

Before the experiments, the animals were left in (B), a large volume of solution for 10 or 15 hours so that they might become in osmotic equilibrium with the solution, and unhealthy animal is easily detected and eliminated from the experiments. Then the animals were taken from (B), the chemical composition, the freezing point depression, and the electric conductivity of the solution therein being measured, (ordinary sea water, diluted or concentrated sea water) and put in (A) or (A′), in the same sea water, diluted or not diluted with distilled water or concentrated by evaporation according to the purpose of the experiment. The volume of the experimental medium was ca. 12 L. . . . . 12 L. which were so large that the concentration of the medium was unchanged in spite of exchanges of water and ions between the medium and the animals. The dilution of the sea water by the addition of distilled water to 3/5 (3 parts of sea water and 2 parts of distilled water) is about the limit of the dilution of the sea water, since in the medium diluted to one half, haemolysis of most of red corpuscles takes place.

The freezing point depression, the chlorine ion concentration, and the electric conductivity were measured by the same methods as in IV. PH was determined colorimetrically, 5 c.c. of the solution being used. The value was corrected for the salt error.

One or two animals were used for a single determination. Swelling or shrinking of the animal was measured by weighing after the animal was wiped by a dry towel. In the case of the permeability of the whole-body-surface, the body weight was measured after the animal exhaled as much water as possible from the respiratory trees; the exhaled sand was filtered on a glass-filter and weighed. The body weight which is described as such, is the sum of the two. In the case of the permeability of the body wall, as described above, a centrifuge tube was tied to the tail of the animal. Therefore, in this case, the body weight means total weight

—(centrifuge + cotton string).

The volume of the inner fluid of the animal, which consists, for the most part, of the body fluid and of a small quantity of the fluid in the alimentary canal was determined as follows: first the total body was weighed and then the body wall was cut, and the inner organs and sand with the alimentary canal were removed and weighed after their containing water was removed as much as possible by means of a glass-filter and a filter pump. The body wall was weighed after being wiped by a dry towel. From the total body weight the weight of the inner organs, sand, and body wall was subtracted and the remaining weight was divided by the specific gravity of the inner fluid. Since the chlorine content of the body fluid is very similar to that of the fluid in the alimentary canal (see V, C, 1, c and Table XVIII) and the specific gravity of the body fluid containing corpuscles is equal to that of sea water of same chlorine ion content within the limit of error (10 c.c. of the body fluid containing 9% corpuscles weigh 10.251 grams at 12.7 C while the same volume of the equilibrium sea water weighs 10.247 grams; 10 c.c. of the body fluid containing 16% corpuscles weigh 10.298 grams at 16.7 C. when the equilibrium sea water weighs 10.237 grams.), so was obtained the specific gravity of the body fluid, though not exactly, from the relation between salinity, specific gravity, and chlorine ion content per c.c., i. e., by means of the A. SCHUMACHER's graphical determination of specific gravity of sea water from t° (C) and S (‰) (1922) and the KNUDSEN's relation: S ‰ = 0.030 + 1.8050 Cl, where S ‰ means salinity of the water, and Cl is the weight of chlorine ion in grams per 1000 grams of sea water. As most of the body fluids have about 5% corpuscles, so was designated the 95% of the last quotient as the fluid part of the body fluid.

After each experiment, some animals were returned to ordinary sea water and sand. Normal sand burrowing took place as usual; they were healthy. Some animals for the body-wall-permeability could not dig sand, not due to their unhealthiness but to the fact that parts of the bodies used for sand intrusion had long been tied with cotton strings.

In order to study the swelling or shrinking of the animal, it is necessary to determine in the first place whether or not the animal, as it rests in the normal sea water which is in equilibrium with the animal, has the constant body weight for the number of minutes required for the experiment.

The data are given in Tables V, VI and, Figs. III, IV indicating that the body weight is practically constant, especially in the case of body-

TABLE V. — The Body Weight of *Caudina* put in the Normal Sea Water, its Mouth and Anus being outside the Sea Water. (Fig. III).

Sep 18, 1930; Medium temp. = 20° ± 1°C.; Cl′ in sea water = 1.90 grs./100 c.c. Cl′ in body fluid = 1.91 grs./100 c.c.

Animal No.	Time in mins.	Body weight in grs.
I	0	40.6
	52	40.6
	149	41.1
	249	40.8
	388	40.8
	527	40.5
	653	40.6
	797	40.4
	900	40.0
	1217	41.4
II	0	45.6
	60	45.6
	150	45.6
	245	45.7
	358	45.8
	501	46.1
	646	46.1
	790	46.1
	892	45.9
	1222	45.8

TABLE VI. — The Body Weight of *Caudina* in the Normal Sea Water, whole Body being put in it. (Fig. IV).

Aug. 16–17, 1929; Medium temp. = 26° ± 1°C.

Animal No.	Time in mins.	Body weight in grs.
I	0	35.0
	10	34.8
	36	34.6
	60	34.1
	85	33.3
	125	34.3
	245	34.3
	305	34.5
	365	34.9
	445	35.2
II	0	18.7
	75	18.6
	240	17.3
	295	17.7
	420	17.2
	1105	17.4

Animal No.	Time in mins.	Body weight in grs.
III	0	22.5
	70	22.5
	190	22.8
	285	22.8
	385	23.0
	1140	23.0
IV	0	26.5
	120	26.4
	280	27.0
	340	26.5
	440	27.8
	1160	26.1

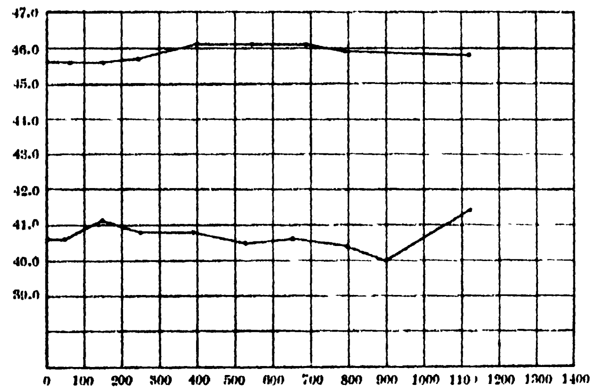


Fig. III. Constancy of body weight of *Caudina* in ordinary sea water, its mouth and anus being outside the medium (Table V).
Ordinate: Body weight of *Caudina* in grs.
Abcissa: Time in mins.

wall-permeability. It is very difficult to weigh *Caudina* correctly, in the case of the whole-body-surface-permeability, owing to its inhalation and exhalation of water (respiration) which sometimes amounts even to 1/4 of its body weight. In addition to this *Caudina* sometimes discharges the body fluid. So that during the course of the experiment on the constancy of the body weight, its discharging had to be watched.

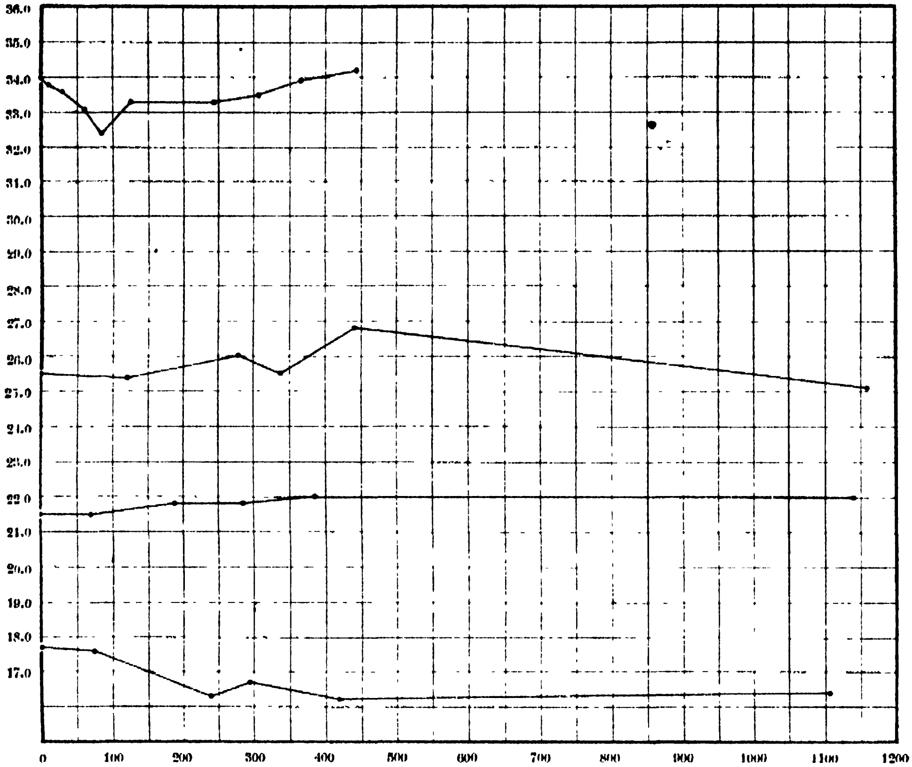


Fig. IV. Constancy of body weight of *Caudina* in ordinary sea water, its body, as a whole, being put in the medium (Table VI).

Ordinate: Body weight of *Caudina* in grs.

Abcissa: Time in mins.

C. Results and Discussion.

1. Osmotic equilibration across the body wall, mouth and anus being outside the experimental solution or body-wall-permeability.

a. The experiments on swelling or shrinking of the animal. The results are given in Table VII and Fig. V, showing swelling or shrinking of animal, *i. e.* increase or decrease in its body fluid in which swelling or shrinking of other organs, for example, body wall is contained (see Tables XXIII, XXIV).

TABLE VII. — Volume or Weight Change of *Caudina* caused by Transport of Water Across the Body Wall (Mouth and Anus being excepted) (Fig. V).

July 29, 1931. Room temp. = 21°C.
Thermostat temp. = 22°±0.02°C.
Initial body fluid = 13.5 grs.

(I) Initial medium in equlibrium with the animals = sea water (pH = 8.3; Cl′ = 1.90 grs./100 c.c.; Vol. = 11.7 L.)
Experimental medium = sea water diluted to 3/5 (pH = 8.2; Cl′ = 1.14 grs./100 c.c.; Vol. = 11.7 L.)

Time in mins.	Body weight without sand & with water in the centrifuge tube in grs.	Water in the centrifuge tube in c.c.	Cl′ in grs./100 c.c. body fluid
0	28.9	0	1.90
25	29.4	0.1	
68	29.7	0.1	
206	30.9	0.1	
351	32.1	0.25	
529	32.0	0.15	
724	32.3	0.20	
831	32.2	0.2	1.17

July 30, 1931. Thermostat temp. = 22°±0.02°C.
Initial body fluid = 17.0 grs.

(II) Initial medium in equilibrium with the animals (contact hours = 24 h.) = the experimental medium in the Experiment I.
Experimental medium = sea water (pH = 8.25; Cl′ = 1.90 grs./100 c.c.; Vol. = 11.7 L.)

0	31.0	0	1.14
27	30.7	0.3	
97	29.8	0.5	
161	28.9	"	
250	28.2	"	
450	27.6	"	
595	27.3	"	
921	26.9	0.55	1.85

July 30, 1931. Initial body fluid = 27.3 grs.

(III) The same condition as in Experiment II.

0	43.0	0	1.14
39	42.4	0.6	
105	41.7	0.8	
171	40.7	0.9	
262	39.8	1.1	
464	38.8	"	
611	37.6	"	
931	37.3	1.2	
1259	37.0	1.25	1.85

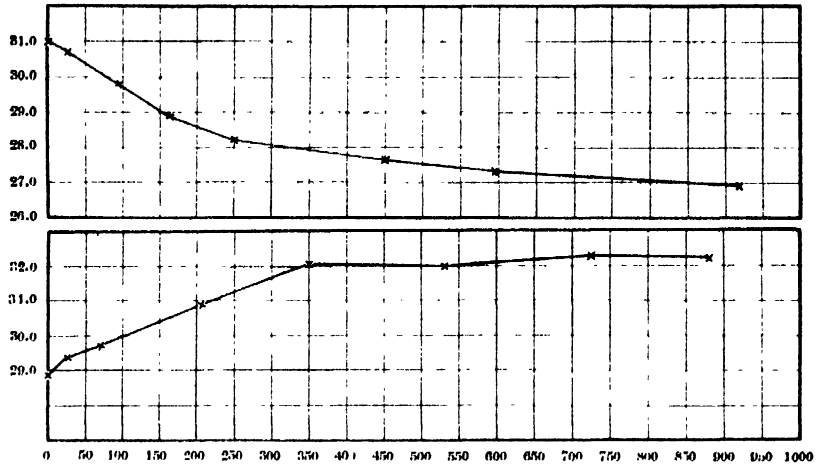


Fig V. Swelling and shrinking of *Caudina*, its mouth and anus being outside the medium (Table VII).

Ordinate: Body weight of *Caudina* in grs Abcissa: Time in mins.

b. To what extent does the monomolecular equation fit the data?

The following groups of the experiments were designed to answer the question, to what extent does the monomolecular equation fit the data in the case of permeability of the body wall? The experiments of this character are summarized in Tables VIII-XVIII or their compiled Table XIX and Figs. VI_A, VI_B.

TABLE VIII. — Chlorine Ion Concentration of the Body Fluid.

I. Aug. 23, 1930 Temp. = 27°±1°C

Initial medium - normal sea water (Cl′ - 1.80; pH - 8.2)

Experimental medium - diluted sea water (Cl′ - 1.40; pH - 8.2-8.0)

Time in mins.	Concentration of chlorine ion in grs. per 100 c.c. of the body fluid.	$0.4343\ K' = \frac{1}{t} \log \frac{C_0 - C_m}{C - C_m}$
0	1.80	—
25	1.76	0.0018
70	1.71	0.0016
152	1.65	0.0013
235	1.55	0.0018
320	1.52	0.0016
392	1.50	0.0015
437	1.49	0.0015

II. Aug. 25, 1930. Temp. = 27°±1°C

Initial medium - normal sea water (Cl′ = 1.79; pH = 8.2)

Experimental medium - diluted sea water (Cl′ = 1.05; pH - 8.2-8.0)

0	1.790	—
24	1.73	0.0015
64	1.64	0.0015
116	1.494	—
210	1.367	0.0017
311	1.25	0.0018
412	1.20	0.0017
528	1.14	0.0017
657	1.10	0.0018
819	1.08	0.0017
942	1.06	—
1072	1.06	—

III. Aug. 31, 1930. Temp.=27°±1°C.
Initial medium=normal sea water (Cl′=1.88; pH=8.2)
Experimental medium=Concentrated sea water (Cl′=2.19; pH=8.2–8.0)

0	1.88	—
20	1.91	0.0022
45	1.94	0.0021
86	2.00	0.0025
140	2.04	0.0025
228	2.10	0.0024
320	2.11	0.0018
415	2.14	—
528	2.17	—
688	2.187	—

Table IX. — Concentration of Chlorine Ion of the Body Fluid. Parenthesis in the Column Indicates the Concentration of the Fluid in the Intestine.

Initial medium=Normal sea water.
Experimental medium=Sea water diluted to 4/5.
(A) Aug. 2–3, 1931. Temp.=27°±0.04°C.
Initial medium=normal sea water (Cl′=1.88; pH=8.2)
Experimental medium=diluted sea water (Cl′=1.52; pH=8.2–8.0)

Time in mins.	Initial body weight with sand in grs.	Initial inner fluid in c.c.	Concentration of chlorine Ion in grs per 100 c.c. of the body fluid	$0.4343\,K' = \frac{1}{t}\log\frac{C_0 - C_m}{C - C_m}$
0	—	—	1.88	—
32	18.20	11.0	1.85	0.0012
32	34.79	14.3	1.85	0.0012
60	16.94	8.0	1.81	0.0016
60	43.77	27.2	1.81	0.0016
122	51.30	27.5	1.76	0.0014
132	24.89	12.1	1.78	0.0011
132	35.99	19.1	1.76	0.0013
240	23.92	14.2	1.71	0.0012
240	38.18	19.5	1.68	0.0015
360	28.38	14.2	1.61	0.0017
360	34.91	16.8	1.63	0.0014

{482	25.29	11.8	1.60	0.0014
{482	45.97	21.9	1.62	0.0012
{600	30.13	—	—	—
{600	47.64	27.4	1.57	0.0014

(B) Oct. 26, 1931. Temp. =27°±0.04°C.
Initial medium = normal sea water (Cl′=1.878; pH=8.2)
Experimental medium = diluted sea water (Cl′=1.508; pH =8.2 8.1)

0			1.878	—
40			1.835	0.00134
60			1.818(1.83)	0.00128
120			1.766	0.00131
182			1.712	0.00142
310			1.649	0.00135
480			1.592(1.59)	0.00132

(C) Nov. 22, 1931. Temp.=27°±0.04°C.
Initial medium = normal sea water (Cl′=1.880)
Experimental medium = diluted sea water (Cl′=1.510)

0			1.880	—
32			1.840(1.86)	0.00166
62			1.809(1.85)	0.00149
120			1.764(1.75)	0.00137
240			1.668(1.69)	0.00153
440			1.580(1.59)	0.00162

TABLE X.—Concentration of Chlorine Ion of the Body Fluid (Fig. VI_B=Table X, A).

Parenthesis indicates the concentration of the fluid in the intestine.

Initial medium = normal sea water.
Experimental medium = sea water diluted to 3/5.
(A) Aug. 5-7, 1931. Temp.=27°±0.04°C.
Initial medium = normal sea water (Cl′=1.90; pH=8.2)
Experimental medium = diluted sea water (Cl′=1.16; pH=8.2-8.0)

Time in mins.	Initial body weight with sand in grs.	Initial inner fluid in c.c.	Concentration of chlorine ion in grs. per 100 c.c. of the body fluid	$0.4343\ K'=\frac{1}{t}\log\frac{C_0-C_m}{C-C_m}$
0	0	0	1.90	—
{30	19.5	11.2	1.84	0.0012
{30	42.1	25.4	1.84	0.0012
{64	38.2	23.2	1.79	0.0011
{64	49.5	31.4	1.80	0.0010
140	47.6	31.4	1.70	0.0010
{240	34.1	19.3	1.53	0.0013
{240	51.1	27.8	1.53	0.0013
{420	28.4	15.6	1.45	0.0010
{420	53.6	31.6	1.41	0.0012
{600	37.6	17.9	1.32	0.0012
{600	46.8	26.1	1.36	0.0010

{840	58.4	37.7	—	—
{840	33.8	17.8	1.261	0.0011
1080	62.8	41.6	1.230	0.0010

(B) Oct. 16, 1931. Temp.=27°±0.04°C.
Initial medium=normal sea water (Cl′=1.863; pH=8.2)
Experimental medium=diluted sea water (Cl′=1.171; pH=8.2 8.0)

0			1.863	—
40			1.761 (1.79)	0.00173
60			1.750	0.00129
120			1.606	0.00168
180			1.570 (1.609)	0.00133
480			1.298	0.00153

(C) Nov. 4, 1931. Temp.=27°±0.04°C.
Initial medium=normal sea water (Cl′=1.864; pH=8.2)
Experimental medium=diluted sea water (Cl′=1.136; pH=8.2-8.0)

0			1.864	—
32			1.806	0.00113
70			1.716	0.00141
120			1.644	0.00130
300			1.414	0.00139
410			1.342	0.00134

TABLE XI.—Concentration of Chlorine Ion of the Body Fluid (Fig. VI$_A$=Table XI, A). Parenthesis in the Column Indicates the Concentration of the Fluid in the Intestine.

Initial medium=sea water diluted to 4/5.
Experimental medium=normal sea water.
(A) Aug. 9–10, 1931. Temp.=27.°45±0.04°C.
Initial medium=diluted sea water (Cl′=1.327; pH=8.2 8.0)
Experimental medium=normal sea water (Cl′=1.863; pH=8.2)

Time in mins.	Initial body weight with sand in grs.	Initial inner fluid in c.c.	Concentration of chlorine ion in grs. per 100 c.c. of the body fluid	$0.4343\ K' = \frac{1}{t} \log \frac{C_0 - C_m}{C - C_m}$
0	—	—	1.324	—
{35	32.8	19.9	1.400	0.0019
{35	52.5	33.4	1.400	0.0019
{75	37.7	22.2	1.496	0.0022
{75	60.8	43.0	1.477	0.0019
{120	29.6	17.3	1.578	0.0023
{120	49.1	29.6	1.548	0.0019
{240	39.2	21.7	1.681	0.0020
{240	50.1	31.7	1.681	0.0019
{420	34.7	20.6	1.800	0.0022
{420	52.7	39.0	1.759	0.0017
600	63.6	41.2	1.810	0.0017

(B) Oct. 27, 1931. Temp.=27° ± 0.04 C.
Initial medium = diluted sea water (Cl′ = 1.516)
Experimental medium = normal sea water (Cl′ = 1.883)

0			1.516	—
35			1.571 (1.550)	0.0020
60			1.600	0.0019
136			1.677	0.0018
180			1.720	0.0020
305			1.786	0.0019
475			1.845 (1.81)	0.0021

TABLE XII. — Concentration of Chlorine Ion of the Body Fluid. Parenthesis in the Column Indicates the Concentration of the Fluid in the Intestine.

Initial medium = sea water diluted to 3/5.
Experimental medium = normal sea water.
Oct. 17, 1931. Temp. = 27° ± 0.04°C
Initial medium = dilute sea water (Cl′ = 1.195)
Experimental medium = normal sea water (Cl′ = 1.863)

Time in mins.	Concentration of chlorine ion of grs. per 100 c.c. of the body fluid	$0.4343\,K' = \frac{1}{t}\log\frac{C_0 - C_m}{C - C_m}$
0	1.195	—
30	1.304	0.0027
71	1.389	0.0021
124	1.459 (1.43)	0.0018
180	1.561 (1.52)	0.0019
302	1.719	0.0022
527	1.809	0.0021

TABLE XIII. — Concentration of Chlorine Ion of the Body Fluid. Parenthesis in the Column Indicates the Concentration of the Fluid in the Intestine.

Initial medium = sea water diluted to 3/5.
Experimental medium = sea water diluted to 4/5.
(A) Nov. 2, 1931. Temp. = 27° ± 0.05°C.
Initial medium = diluted sea water (Cl′ = 1.159)
Experimental medium = less diluted sea water (Cl′ = 1.459)
Animals were kept 15 hours in the initial medium before the experiment.

Time in mins.	Concentration of chlorine ion in grs. per 100 c.c. of the body fluid	$0.4343\,K' = \frac{1}{t}\log\frac{C_0 - C_m}{C - C_m}$
0	1.159	—
72	1.253 (1.233)	0.0023
133	1.279	0.0017

182	1.362	0.0027
300	1.387 (1.368)	0.0021
460	1.425	0.0021

(B) Nov. 5, 1931. Temp. =27°±0.05°C.
Initial medium = diluted sea water (Cl′ = 1.167)
Experimental medium = less diluted sea water (Cl′ = 1.500)

0	1.167	—
30	1.220 (1.191)	0.0025
62	1.259 (1.210)	0.0023
121	1.318 (1.25)	0.0022
256	1.413	0.0023
460	1.456	0.0019

TABLE XIV. — Concentration of Chlorine Ion of the Body Fluid. Parenthesis in the Column Indicates the Concentration of the Fluid in the Intestine.

Initial medium = normal sea water,
Experimental medium = concentrated sea water.*

(A) Nov. 14, 1931. Temp. =27°±0.05°C.
Initial medium = normal sea water (Cl′ = 1.875; pH = 8.2)
Experimental medium = concentrated sea water (Cl′ = 2.647; pH = 8.2–8.0)

Time in mins.	Concentration of chlorine ion in grs per 100 c.c. of the body fluid	$0.4343\ K' = \frac{1}{t} \log \frac{C_0 - C_m}{C - C_m}$
0	1.875	—
30	2.003	0.0026
40	2.043 (2.00)	0.0027
60	2.132	0.0029
120	2.259 (2.22)	0.0025
184	2.385 (2.331)	0.0025
290	2.508	0.0026
410	2.608	0.0032

(B) Nov. 18, 1931. Temp. =27°±0.05°C.
Initial medium = normal sea water (Cl′ = 1.871)
Experimental medium = concentrated sea water (Cl′ = 2.649)

0	1.861	—
30	2.000 (1.93)	0.0028
60	2.081 (1.97)	0.0024
120	2.172	0.0018
245	2.428 (2.37)	0.0023
430	2.543 (2.522)	0.0021

* *Caudina* when placed in the high concentration of the above two experimental solutions for a considerable time shows difficulty in surviving. But survival in those solutions was possible if the mouth and anus were outside the solutions.

TABLE XV. — Concentration of Chlorine Ion of the Body Fluid. Parenthesis in the Column Indicates the Concentration of the Fluid in the Intestine.

Initial medium = concentrated sea water.
Experimental medium = normal sea water.
Nov. 19, 1931. Temp. = 27° ± 0.05°C.
Initial medium = concentrated sea water (Cl′ = 2.264)
Experimental medium = normal sea water (Cl′ = 1.906)

Time in mins.	Concentration of chlorine ion in grs per 100 c.c. of the body fluid	$0.4343\ K' = \frac{1}{t} \log \frac{C_0 - C_m}{C - C_m}$
0	2.259	—
50	2.181 (2.20)	0.0022
120	2.114	0.0019
263	2.013 (2.04)	0.0020
364	1.979 (2.003)	0.0019
543	1.946	0.0023

TABLE XVI. — Concentration of Chlorine Ion of the Body Fluid.

(A) Oct. 2, 1930. Temp. = 17° ± 1°C.
Initial medium = normal sea water (Cl′ = 1.88)
Experimental medium = diluted sea water (Cl′ = 1.50)

Time in mins.	Concentration of chlorine ion in grs. per 100 c.c. of the body fluid	$0.4343\ K' = \frac{1}{t} \log \frac{C_0 - C_m}{C - C_m}$
0	1.88	—
207	1.77	0.00072
330	1.67	0.00106
480	1.63	0.00097
600	1.62	0.00083
922	1.55	0.00096
1008	1.53	0.00109
1267	1.53	0.00087
2100	1.51	0.00075

(B) Oct. 10, 1930. Temp. = 16° ± 1°C.
Initial medium = normal sea water (Cl′ = 1.88)
Experimental medium = diluted sea water (Cl′ = 1.08)

0	1.88	—
150	1.66	0.00093
180	1.56	0.00123
209	1.54	0.00106
435	1.39	0.00095

590	1.37	0.00075
906	1.20	0.00091
1035	1.21	0.00076
1228	1.16	0.00081
1490	1.14	0.00076
1738	1.12	0.00075
2207	1.13	0.00055
2605	1.10	0.00062

TABLE XVII. — Change of Cl′, Δ, κ of the Body Fluid of *Caudina*.

Oct. 29, 1930. Medium temp. = 15°±1°C.

Initial medium = normal sea water (Cl′ = 1.920 (25°±0.0°2C); pH = 8.2; κ = 0.0486 (25°±0.02°C))

Experimental medium = diluted sea water (Cl′ = 1.518 (25°±0.02°C); pH = 8.2–8.1; κ = 0.0397 (25°±0.02°C))

Time in mins.	Concentration of chlorine ion in grs. per 100 c.c. (25°±0.02°C)	Δ	κ (25°±0.02°C)	$0.4343\,K' = \frac{1}{t}\log\frac{C_0-C_m}{C-C_m}$
0	1.919	—	0.0486	—
165 (Mix. from 2 animals)	1.825	—	0.0467	0.00070
360	1.722	—	0.0441	0.00082
780	1.562	—	0.0412	0.00123
1080	1.545	—	0.0407	—
1440	1.532	—	0.0401	—
1800	1.545	—	0.0405	—

Oct. 24, 1930. Medium temp. = 15°±1°C.

Initial medium = normal sea water (Cl′ = 1.91 (15°C); pH = 8.2; Δ = 1.80)

Experimental medium = diluted sea water (Cl′ = 1.54 (15°C); pH = 8.1; Δ = 1.55)

0	1.91	1.89	—	—
120	1.83	1.82	—	0.00088
300	1.76	1.76	—	0.00075
600	1.65	1.63	—	0.00089
900	1.59	1.55	—	0.00097
1230	1.55	1.56	—	—

TABLE XVIII.—Relation between Chlorine Ion Content of the Body Fluid and that in the Alimentary Canal in the Case of Permeability across the Body Wall.

Aug. 13, 1931. Temp.=27°.45±0.04°C.
Initial medium=normal sea water (Cl′=1.764; pH=8.2)
Experimental medium=diluted sea water (Cl′=1.130; pH=8.2–8.1)

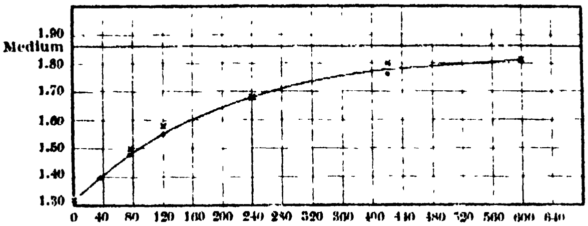
Time in mins.	Concentration of chlorine ion in grs. per 100 c.c. of body fluid	Concentration of chlorine ion in grs. per 100 c.c. of fluid in alimentary canal	$0.4343\ K' = \frac{1}{t} \log \frac{C_0 - C_m}{C - C_m}$
0	1.760	1.760	—
35	1.690	1.713	0.0015
91	1.628	1.636	0.0012
150	1.537	1.565	0.0013
240	1.484	1.484	0.0011
418	1.349	1.380	0.0011
596	1.293	1.297	0.0010
894	1.209	1.218	0.0010

TABLE XIX (compiled table from Tables IX–XVI). 0.4343 K′ is from the Mean of One Series of Experiments (Tables IX–XVI).

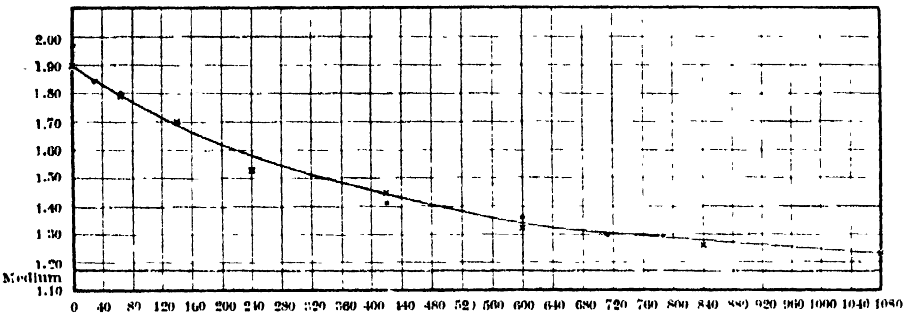
	Date	$C_0 \times 100$	$C_m \times 100$	0.4343 K′ 27°C.
Course of swelling	Aug. 2–3, '31	1.88	1.52	0.0014
	Oct. 26, '31	1.878	1.508	0.0013
	Nov. 22, '31	1.880	1.510	0.0015
	Aug. 2–7, '31	1.90	1.16	0.0011
	Oct. 16, '31	1.863	1.171	0.0015
	Nov. 4, '31	1.864	1.136	0.0013
	* Nov. 19, '31	2.264	1.906	0.0021
Course of shrinking	Nov. 14, '31	1.875	2.647	0.0027
	Nov. 18, '31	1.871	2.649	0.0023
	Aug. 9–10, '31	1.327	1.863	0.0020
	Oct 27, '31	1.516	1.863	0.0020
	Oct. 17, '31	1.195	1.863	0.0021
	Nov. 2, '31	1.159	1.459	0.0022
	Nov. 5, '31	1.167	1.500	0.0022

* This greatness of 0.4343 K′, I think, does not depend on the greatness of C_m but on the negligibility of elasticity.

A



B



Figs VIA–VIB. Change of concentration of body fluid of *Caudina*, its mouth and anus being outside the medium (Table XI, A; Table X, A). Ordinate, concentration of Cl′ in grs. per 100 c.c. of body fluid; abcissa, time in mins.

A. Concentrated medium (ordinary sea water).

B. Diluted medium (diluted sea water).

They show that Cl′, Δ and x of the body fluid change in the same direction, parallel with each other and come to nearly the same values as those of the external medium, and the monomolecular equation fits approximately the data. Moreover there is no effect of salt concentration of the medium on the permeability factor, K′ at least under these conditions (see f, iii). It is observed, however, that the value of K′ for shrinking (exit of the water and entrance of ions) is much greater than that for swelling (entrance of water and exit of ions). This relation was also derived by B. LUCKÉ, H. K. HARTLINE, and M. McCUTCHEON (1931) in the ARBACIA egg from their equation. However the swelling of the animal, previously shrunken in concentrated medium, in ordinary sea water (Table XV; Table XIX) has the same K′ as that for shrinking, indicates that it is not identical with the socalled "irreciprocal permeability" in the skin membrane of frogs (REID (1890); WERTHEIMER (1923)), but is probably due to the fact that other forces such as elastic which is responsible for

it was neglected in deriving the equation as has been suggested by B. LUCKÉ and others. To illustrate this point I have only one series of experiments.

c. Assumptions (3) and (4).

Aussumption (3): The concentrations of the body fluid and the surroundings are kept uniform. The time required to equalize the concentration difference within the body of the animal, across the alimentary tract between the body fluid and the solution in the alimentary canal, is neglected as compared with that required for the water and (ions) to diffuse across the body wall.

This is, indeed, the case to some extent as shown in Tables XVIII and IX–XV (see also V, C, 1, e).

Assumption (4): The volume of the surrounding medium is so large that the concentration of each ion is unchanged in spite of exchanges of water and ions between the medium and the body fluid.

As previously mentioned, M. YAZAKI observed the circulation of the body fluid and outer medium was mechanically stirred and its volume was so large that it served the purpose (V. B).

d. Assumption on integration of Equation (5): The permeability surface of the body wall of animal divided by the volume of the inner fluid, *i. e.* $\frac{S}{V}$ is constant.

It is given in Table XX.

TABLE XX. — Relation between the Body Surface and the Volume of the inner Fluid in the Case of Permeability of the Body Wall.

Aug. 23, 1931. Medium = normal sea water.

Animal No.	Body weight with sand in grs.	Body volume in c.c.	Body surface immersed in the medium in cm^2 (S)	Body wall in grs. (S′)	Inner fluid in c.c. (V)	$\frac{S}{V}$	$\frac{S'}{V}$
I	69.0	58.0	84	10.6	31.6	2.6	0.33
II	42.7	39.8	65	11.6	22.8	2.8	0.57
III	41.3	39.5	62	9.1	27.9	2.2	0.32
IV	37.9	33.0	53	7.7	21.5	2.5	0.36
V	32.6	29.8	48	8.6	18.4	2.7	0.47
VI	21.3	19.0	31	6.3	9.9	3.2	0.64

It shows that in the case of body wall permeability, the experimental condition is so arranged that $\frac{S}{V}$, *i. e.* permeability surface per unit volume of the inner fluid is practically constant while $\frac{S'}{V}$ is not constant, thus satisfying the assumption to some measure.

Data throughout the course of osmotic change are wanting.

e. Relation between the quantities of water and ions transported in the case of permeability of body wall.

Whatever osmotically may happen, the total amount of salts in the inner fluid, *i. e.*, the product of concentration and volume of the fluid must be constant if the body wall of *Caudina* is permeable to water but not to salts. Then we may write in this case, $CV=U$ where C is the concentration of salts in solution in the inner fluid, V is the volume of the fluid, and U is a constant. The change of concentration of the inner fluid must, in this case, directly correspond to the gain or loss of water in the inner fluid. If the body wall is also permeable to ions even a little, CV can never be constant. As a matter of fact smaller discrepancies are shown in the second column from the last in Tables XXI–XXII.

This fact, together with other experiments on the permeability of body wall to ions (Tables XXXII, XXXIII), would lead to the conclusion that the surface of this animal is not only permeable to water but also to salts in solution in the sea water, though it might be very slight.

On the determination of the increase of water in the body cavity in the case of swelling (for example) must be remarked the following sources of errors; (1) The body wall and inner organs also swell and therefore increase in their weights (Tables XXIII, XXIV); (2) sand in the alimentary tract, after being filtrated, still contains water (ca. 8%) which was at most 2% of the total volume in the experiment (see Tables XXI, XXII); (3) the concentration of chlorine ion in the alimentary tract is practically very similar to, but, more accurately stated, may be higher than that of the body cavity at most by 5% (see IX–XV, XVIII), and the volume of the solution in the intestine was at most 1/3 of total inner fluid and therefore the error introduced in appoximate calculations of $(CV)_F$ by applying the concentration of body fluid for C was only about one %. The errors produced from the second and third conditions make the 2nd column from the last in Tables XXI–XXII, *i. e.*, $(CV)_I-(CV)_F$ slightly larger while that from the first condition makes it slightly smaller.

In the last column in Tables XXI–XXII it is seen that the numerical

TABLE XXI. — Relation between the Quantities across the Body Wall in the Case of

Aug. 9, 1931. 27.45°±0.05°C.
Course of shrinking.
Initial medium ($C_i = Cl' = 1.327$; $\sigma_i = 1.016$ at 20°C)
Experimental medium ($Cl' = 1.863$)

Time (mins.)	Body weight with sand (grs.) Initial	Final	Body wall (grs.)	Sand (grs.)	Viscera (grs.)	Body wall + sand + viscera (grs.)	Fluid in tube (c.c.) Initial V_1'	Final V_2'	Inner fluid (grs.) Initial W_1	Final W_2	W_1-W_2
0											
35	32.8	31.5	8.1	2.0	1.4	11.5	0.5	1.4	21.3	20.0	1.3
35	52.5	51.8	12.6	1.1	3.1	16.8	0	0	35.7	35.0	0.7
75	37.7	36.5	10.0	1.8	2.1	13.9	0	0	23.8	22.6	1.2
75	60.8	58.7	10.8	1.4	2.6	14.8	0.1	0.6	46.0	43.9	2.1
120	29.6	28.0	6.7	0.6	3.8	11.1	0	0.1	18.5	16.9	1.6
120	49.1	45.2	9.8	2.9	4.7	17.4	0	2.6	31.7	27.8	3.9
240	39.2	35.7	11.8	0.4	3.9	16.1	0.2	0.95	23.1	19.6	3.5
240	50.1	45.6	12.7	0.1	3.4	16.2	0.5	0.9	33.9	29.4	4.5
420	34.7	30.9	10.5	1.9	1.4	13.8	0.1	1.25	20.9	17.1	3.8
600	63.6	55.5	14.8	0.8	3.9	19.5	0.8	2.1	44.1	36.0	8.1

TABLE

A. Aug. 5–7, 1931. 27°C.
Course of swelling.
Initial medium ($C_i = Cl' = 1.90$; $\sigma_i = 1.024$ at 20°C)
Experimental medium ($Cl' = 1.16$)

Time (mins.)	Body weight with sand (grs.) Initial	Final	Body wall (grs.)	Sand (grs.)	Viscera (grs.)	Body wall + sand + viscera (grs.)	Fluid in tube (c.c.) Initial V_1'	Final V_2'	Inner fluid (grs.) Initial W_1	Final W_2	W_1-W_2
0											
30	19.5	20.0	5.7	1.0	1.3	8.0	0	0	11.5	12.0	-0.5
30	42.1	40.7	10.1	2.7	3.3	16.1	4.2	5.8	26.0	24.6	1.4
64	38.2	38.6	10.5	1.2	2.7	14.4	0.2	1.0	23.8	24.2	-0.4
64	49.5	51.1	13.4	0.4	3.6	17.4	0.3	0.45	32.1	33.7	-1.6
140	47.6	49.4	10.0	2.8	2.7	15.5	0.1	0.4	32.1	33.9	-1.8
240	34.1	37.1	9.3	3.4	1.6	14.3	0	0.6	19.8	22.8	-3.0
240	51.1	54.7	12.7	5.9	4.0	22.6	0	0.5	28.5	32.1	3.6
420	28.4	31.5	8.2	2.1	2.1	12.4	0	0	16.0	19.1	-3.1
420	53.6	59.4	12.0	6.0	3.3	21.3	0	0.9	32.3	38.1	-5.8
600	37.6	41.7	11.6	4.1	3.6	19.3	0	0	18.3	22.4	-4.1
600	46.8	51.3	11.6	4.9	3.6	20.1	0.15	2.1	26.7	31.2	-4.5
840	33.8	35.2	10.8	1.4	3.3	15.5	0	4.2	18.3	19.7	-1.4
1080	62.8	69.1	13.2	4.8	2.2	20.2	1.0	4.5	42.6	48.9	6.3

B. Oct. 2, 1930. 17°±1°C.
Course of swelling.
Initial medium ($C_i = Cl' = 1.88$; $\sigma_i = 1.023$ at 20°C)
Experimental medium ($Cl' = 1.50$)

Time (mins.)	Body weight with sand (grs.) Initial	Final	Body wall (grs.)	Sand (grs.)	Viscera (grs.)	Body wall + sand + viscera (grs.)	Fluid in tube (c.c.) Initial V_1'	Final V_2'	Inner fluid (grs.) Initial W_1	Final W_2	W_1-W_2
0											
150							0	0	22.3	23.3	-1.0
180							0	0	20.0	20.2	-0.2
209							0	0	13.3	14.5	-1.2
435							0	0	23.5	25.0	-1.5
590							0	0	17.8	20.9	-3.1
906							0	4.1	12.3	12.6	-0.3
1035							0	8.0	19.9	14.4	5.5
1228							0	0.9	11.2	12.6	-1.4
1490							0	0	12.7	15.8	-3.1
1738							0	5.2	13.4	12.0	1.4
2208							0	4.5	14.1	13.6	0.5
2605							0	2.9	15.4	14.0	1.4

of Water and Ions Transported
Body-Wall-Permeability.

Concentration of chlorine ion in grs. per 100 c.c. Body fluid C_2	Concentration of chlorine ion in grs. per 100 c.c. Fluid in tube C_2'	Specific gravity of inner fluid σ_2	Inner fluid (c.c.) Initial $V_1=\frac{W_1}{\sigma_1}\times 0.95$	Inner fluid (c.c.) Final $V_2=\frac{W_2}{\sigma_2}\times 0.95$	$\Delta V=V_2-V_1$ or $(V_2+V_2')-(V_1+V_1')$	$(CV)_I=C_1(V_1+V_1')$	$(CV)_F=C_2V_2+C_2'V_2'$ $=C_2V_2+C_1V_2'$	$(CV)_I-(CV)_F$	Total Ion diffused in (or out), Water diffused out (or in), in gramions / in grammols = Δg in gramions / ΔV in grammols = $(CV)\times\frac{1}{35.46}\times\frac{1.11}{0.54} \div \Delta V\times\frac{1}{18}$ or $\frac{k_2}{k_1}$
1.324		1.016							
1.400	—	1.017	19.9	18.7	—	27.0	28.0	-1.0	—
1.400	—	1.017	33.4	32.7	-0.7	44.2	45.8	-1.6	0.024
1.496	—	1.018	22.3	21.1	1.2	29.5	31.6	-2.1	0.018
1.477	—	1.018	43.0	41.0	-1.5	57.1	61.4	-4.3	0.030
1.578	-	1.020	17.3	15.7	-1.6	22.9	24.9	-2.0	0.013
1.548	1.36	1.020	29.7	25.9	—	39.3	43.6	-4.3	—
1.681	—	1.021	21.6	18.2	-2.7	28.9	31.9	3.0	0.012
1.681	·	1.021	31.7	27.4	-3.9	42.6	47.3	4.7	0.013
1.800	1.69	1.023	19.5	15.9	—	26.0	30.8	-4.8	—
1.810	1.68	1.023	41.2	33.4	7.5	56.9	64.0	8.9	0.012

XXII.

1.90		1.024							
1.84	—	1.023	10.7	11.1	—	20.3	20.4	-0.1	—
1.84	—	1.023	24.1	22.8	—	53.8	53.0	0.8	—
1.79	—	1.022	22.1	22.5	—	42.4	42.2	0.2	—
1.80	—	1.023	29.8	31.3	—	57.2	57.2	0.	—
1.70	—	1.021	29.8	31.5	2.0	56.8	54.3	2.5	0.013
1.53	—	1.019	18.4	21.3	3.5	35.0	33.7	1.3	0.004
1.53	··	1.019	26.4	29.9	4.0	50.2	46.7	3.5	0.009
1.45	—	1.018	14.9	17.8	2.9	28.3	25.8	2.5	0.009
1.41	—	1.017	30.0	35.6	6.5	57.0	51.9	5.1	0.008
1.32	—	1.016	17.0	20.9	3.9	32.3	27.6	4.7	0.013
1.36	1.42	1.017	24.8	29.2	6.4	47.4	42.7	4.7	0.008
1.26	1.26	1.015	17.0	18.4	5.6	32.3	28.5	3.8	0.007
1.23	1.30	1.015	39.5	45.8	9.8	77.0	62.2	14.8	0.016

1.88		1.023							
1.66	—	1.020	20.7	21.7	1.0	38.9	36.0	2.9	0.030
1.56	—	1.019	18.6	18.8	0.2	34.9	29.4	5.5	—
1.64	—	1.019	12.4	13.5	1.1	23.2	20.8	2.4	0.023
1.39	—	1.017	21.8	23.8	2.0	41.0	33.1	7.9	0.041
1.37	—	1.017	16.5	19.5	3.0	31.1	26.8	4.3	0.015
1.20	1.29	1.015	11.4	11.8	—	21.5	19.5	2.0	—
1.21	1.83	1.015	18.5	13.5	—	34.8	31.0	3.8	—
1.16	1.81	1.014	10.4	11.8	—	19.6	15.3	4.3	—
1.14	—	1.014	11.8	14.8	3.0	22.2	16.9	5.3	0.019
1.12	1.20	1.013	12.5	11.3	4.0	23.4	18.9	4.5	0.012
1.13	1.15	1.014	13.1	12.8	4.2	24.6	19.6	5.0	0.012
1.10	1.70	1.013	14.3	13.1	—	26.9	19.4	7.5	—

TABLE XXIII. — Shrinking of Body Wall.

After the animals were kept in contact with the solution so as to reach osmotic equilibrium (27°±0.04°C), the body wall was cut and the inner contents were thrown away.

Then only the body wall was returned to the original solution. Weighing of the body wall was made after being wiped with a dry towel.

Oct. 13, 1931. 27.4°±0.04°C.
Initial solution = sea water diluted to 3/5 which was kept in contact with animals for 20 hrs.
Experimental solution = the same diluted sea water.

Time in mins.	Weight of body wall in grs.
0	9.1
10	8.7
30	8.6
60	8.6
125	8.8
240	8.8
290	8.9

Oct. 13, 1931. 27.°4±0.04°C.
Initial solution = sea water diluted to 3/5 which was kept in contact with animals for 15 hours.
Experimental solution = normal sea water.
Before weighing at the time 0, the body wall was left in initial solution for at least 20 minutes.

A	0	7.9
	10	7.3
	30	7.0
	60	6.9
	120	6.8
	240	6.8
	400	6.7
	600	6.5
	630	6.4
B	0	8.9
	10	8.5
	30	8.2
	60	8.0
	133	8.0
	250	7.6
	400	7.6
	560	7.5
C	0	9.6
	10	9.0
	30	8.6
	60	8.3
	120	8.3
	240	8.4
	430	8.0

TABLE XXIV. — Swelling of the Body Wall.

Oct. 14, 1931. 27.4°±0.04°C.

I. Initial solution = normal sea water which was kept in contact with animals for 5 hrs.
Experimental solution = the same sea water.
Before weighing at the time 0, the body wall was left in the solution for 10 minutes.

Time in mins.	Weight of body wall in grs.
0	10.2
10	9.8
30	9.7
60	9.7
120	9.7

Next it was placed in sea water diluted to 3/5.

10	9.8
30	9.9
60	10.0
170	10.4
242	10.6
300	10.7
360	10.8
784	11.3
978	11.3

Oct. 14, 1931. 27.4°±0.04°C.

II. Initial solution = normal sea water which was kept in contact with animals for 6 hours.
Experimental solution = the same sea water.
Before weighing at the time 0, the body wall was left in the solution for 30 minutes.

0	8.6
10	8.3
30	8.3

Next it was placed in sea water diluted to 3/5.

10	8.3
30	8.4
60	8.4
180	8.7
240	8.8
305	9.0
365	9.1
805	9.7
995	9.7

value obtained from

$$\frac{\text{Total ion diffused in (or out) in gramions}}{\text{Water diffused out (or in) in grammols}} = \frac{\text{Cl}' \text{ in grams} \times \frac{1}{35.46} \times \frac{1.11}{0.54}}{\text{Water diffused out (or in) in grammols}}$$

which corresponds to $\frac{k_2}{k_1}$ in Equation (10) (cf. Table XXX) seems to be the order of a few hundredths.

f. Assumption on deriving Equation (7) from Equation (6).

(i) If C in gramions of total ions per unit grammol of water is neglected as compared with $\frac{k_2}{k_1}$, we obtain also (7) from (6). But such assumption is not admissible owing to the same order of magnitude in both C and $\frac{k_2}{k_1}$ as will be seen from the following example; for example

$$C \text{ for } 0.02 \text{ gr. Cl/1 c.c.} = \frac{0.02}{35.46} \times \frac{1.11}{0.54} \times 18 \frac{\text{gramions (Total ions)}}{\text{grammols } (H_2O)}$$

$$= 0.021 \text{ and } \frac{k_2}{k_1} \doteq 0.01 \text{ (Tables XXI–XXII)}$$

(ii) $\dfrac{C+\frac{k_2}{k_1}}{C_0+\frac{k_2}{k_1}}$. It is seen from the example that C expressed in grs. Cl′ per 1 c.c. is practically numerically equal to C expressed in gramions total ions per 1 grammol H_2O. Equation (6) will be written in the form

$$0.4343\,K' = 0.4343\frac{S}{V}(C_m k_1 + k_2) = \frac{1}{t}\log\frac{C_0 - C_m}{C - C_m} + \frac{1}{t}\log\frac{C+\frac{k_2}{k_1}}{C_0+\frac{k_2}{k_1}}.$$

When the two terms of the right hand side of this equation are culculated respectively, we obtain the following results as are shown in Table XXV.

Table XXV shows that the value of 0.4343 K′ of shrinking is much larger than that of swelling whether we neglect $\frac{1}{t}\log\dfrac{C+\frac{k_2}{k_1}}{C_0+\frac{k_2}{k_1}}$ on the calculation or not. In addition to this, it is, as previously seen, difficult to obtain the true value of $\frac{k_2}{k_1}$ from the experiment. So that it may be as well to have used, for the sake of simplicity, the monomolecular equation resulting from the neglection of $\frac{1}{t}\log\dfrac{C+\frac{k_2}{k_1}}{C_0+\frac{k_2}{k_1}}$ on the calculation of the value of 0.4343 K′ in the preceding experiments on the swelling and shrinking of animals.

TABLE XXV.

(1) The course of swelling.
Aug. 2-3, 1931. Temp. = 27° ± 0.04°C.
Initial medium = normal sea water (Cl′ = 1.88)
Experimental medium = diluted sea water (Cl′ = 1.52)

Time in mins.	Concentration of chlorine in grs. per 100 c.c. of the body fluid	$\frac{1}{t}\log\frac{C_0-C_m}{C-C_m}$	$\frac{1}{t}\log\frac{C+\frac{k_2}{k_1}}{C_0+\frac{k_2}{k_1}}$ as $\frac{k_2}{k_1}=0$	as $\frac{k_2}{k_1}=0.01$	as $\frac{k_2}{k_1}=0.02$
0	1.88	—	—	—	—
32	1.85	0.0012	0.00022	-0.00014	-0.00010
32	1.85	0.0012	-0.00022	0.00014	-0.00010
60	1.81	0.0016	-0.00028	-0.00018	0.00013
60	1.81	0.0016	0.00028	0.00018	0.00013
120	1.76	0.0014	-0.00024	-0.00015	0.00011
132	1.78	0.0011	0.00018	0.00012	-0.00009
132	1.76	0.0011	0.00022	-0.00014	-0.00010
240	1.71	0.0013	0.00017	0.00011	0.00007
240	1.68	0.0012	0.00020	0.00013	-0.00010
360	1.61	0.0015	0.00019	-0.00012	0.00009
360	1.63	0.0017	0.00017	-0.00011	-0.00008
482	1.60	0.0014	0.00015	0.00009	0.00007
482	1.62	0.0014	-0.00013	-0.00009	-0.00006
600	1.57	0.0014	-0.00011	0.00008	-0.00006

(2) The course of shrinking.
Aug. 9-10, 1931. Temp. = 27.45° ± 0.04°C.
Initial medium = diluted sea water (Cl′ = 1.327)
Experimental medium = normal sea water (Cl′ = 1.863)

0	1.324	—	—	—	—
35	1.400	0.0019	0.00069	0.00040	0.00028
35	1.400	0.0019	0.00069	0.00040	0.00028
75	1.496	0.0022	0.00071	0.00042	0.00029
75	1.477	0.0019	0.00063	0.00037	0.00026
120	1.578	0.0023	0.00064	0.00038	0.00027
120	1.548	0.0019	0.00057	0.00033	0.00024
240	1.681	0.0020	0.00043	0.00026	0.00018
240	1.681	0.0019	0.00032	0.00026	0.00018
420	1.800	0.0022	0.00032	0.00019	0.00014
420	1.759	0.0017	0.00029	0.00018	0.00013
600	1.810	0.0017	0.00023	0.00014	0.00010

(iii) $0.4343\ K'=0.4343\frac{S}{V}(C_m k_1+k_2)$.

If the values of k_1, k_2 and $\frac{S}{V}$ are fixed in this equation, $0.4343\ K'$ will be large when C_m is large.

In Table XIX, the values of 0.4343 K′, 27°C. are given for a number of experiments, using the same monomolecular equation for

swelling and shrinking. It indicates that they are not always proportional to C_m but vary considerably in different experiments probably due to complexity of animal structure, unequality of condition of animal, elasticity, swelling and shrinking, of the body wall *etc.*

2. Osmotic equilibration across the total body surface including mouth-alimentary-canal, anus-respiratory-trees (whole body placed in the solution).

The results are summarized in Tables XXVI, XXVII, XXVIII, XXIX, Figs. VII_A, and VII_B.

TABLE XXVI. — Concentration of Chlorine Ion of the Body Fluid of *Caudina*, the whole Body being placed in the Medium (Fig. VII).

Aus. 20–21, 1931. Temp. = 27.°45 ± 0.05°C.

A. Initial medium = normal sea water (Cl′ = 1.910 (15°C); pH = 8.2)
Experimental medium = diluted sea water (Cl′ = 1.145 (15°C); pH = 8.1)

Time in mins.	Body weight with sand at the time of fluid collection in grs.	Concentration of chlorine ion of body fluid in grs. per 100 c.c.	Concentration of chlorine ion in intestine in grs. per 100 c.c.	$0.4343K' = \frac{1}{t}\log\frac{C_0 - C_m}{C_t - C_m}$
0	—	1.908	—	—
0	—	1.910	—	—
20	39.1	1.619	1.55	0.0104
20	65.3	1.755	—	0.0049
30	30.0	1.611	—	0.0072
30	67.4	1.725	—	0.0040
56	44.8	1.544	—	0.0050
56	56.9	1.472	—	0.0066
81	31.3	1.379	1.45	0.0064
81	53.0	1.467	—	0.0046
110	44.2	1.387	—	0.0045
110	48.5	1.381	1.38	0.0046
141	43.6	1.242	—	0.0064
155	—	1.350	—	0.0037
210	...	1.250	1.25	0.0041
300	—	1.180	—	—
430	—	1.175	—	—

Aug. 16 17, 1931. Temp. = 27.°45 ± 0.05°C.

B. Initial medium = diluted sea water (Cl′ = 1.21 (15°C); pH = 8.2–8.0)
Experimental medium = normal sea water (Cl′ = 1.85 (15°C); pH = 8.3)

0	—	1.20	—	—
20	21.3	1.425	—	0.0095
20	42.7	1.381	—	0.0070
40	20.3	1.59	—	0.0100
40	25.6	1.59	—	0.0095
40	41.5	1.57	—	0.0091
70	15.9	1.73	—	0.0105
70	27.3	1.77	—	0.0130
70	41.7	1.71	—	0.0095

100	17.1	1.82	—	0.0134
100	39.3	1.71		0.0067
143	39.0	1.80	—	0.0079
143	27.0	1.81	—	0.0084
180	25.0	1.83	—	0.0084
180	37.9	1.83	-	0.0084
241	32.4	1.85	—	—
330	—	1.84	—	—
420	—	1.85	—	—
510	—	1.85	—	—

TABLE XXVII. — Concentration of Chlorine Ion of the Body Fluid, the whole Body being placed in the Medium.

Animals of similar size as possible were selected.

Aug. 1, 1930. Temp. = 26° ± 1°C.
Initial medium = normal sea water (Cl′ = 1.928 (15°C); pH = 8.2)
Experimental medium = diluted sea water (Cl′ = 1.565 (15°C); pH = 8.2-8.1)

Time in mins.	Concentration of chlorine ion of the body fluid in grs. per 100 c.c.	$0.4343\,K' = \frac{1}{t}\log\frac{C_0-C_m}{C-C_m}$
0	1.928	
10	1.868	0.0079
20	1.768	0.0126
40	1.752	0.0061
70	1.747	0.0043
100	1.679	0.0050
160	1.637	0.0044
220	1.583	0.0059
260	1.571	0.0069
310	1.567	—
335	1.574	—

Aug. 14, 1930. Temp. = 26° ± 1°C.
Initial medium = normal sea water (Cl′ = 1.896 (15°C); pH = 8.2)
Experimental medium = diluted sea water (Cl′ = 1.057 (15°C); pH = 8.2-8.0)

0	1.899	—
15	1.753	0.0054
35	1.596	0.0055
67	1.441	0.0051
100	1.340	0.0047
150	1.253	0.0042
218	1.135	0.0048
287	1.150	0.0033
342	1.092	0.0040
395	1.108	0.0031
435	1.070	0.0042
467	1.067	0.0041
538	1.075	0.0049

Aug. 7, 1930. Temp. = 27° ± 1°C.
Initial medium = normal sea water (Cl′ = 1.884 (15°C); pH = 8.2)
Experimental medium = concentrated sea water (Cl′ = 2.367; pH = 8.0)

0	1.884	—
10	1.944	0.0058
45	2.052	0.0041
75	2.151	0.0048
120	2.213	0.0041
180	2.320	0.0056
265	2.344	0.0050
325	2.364	0.0068
285	2.351	0.0057

TABLE XXVIII. — Changes of Cl′, Δ, $\varkappa$ of the Body Fluid of *Caudina*, whole Body being placed in changed Medium.

Oct 31 — Nov. 2, 1930. Medium temp. = 12°±1°C.
Initial medium = normal sea water (Cl′ = 1.905 (25°±0.02°C); pH = 8.2; Δ = 1.91±0.01; $\varkappa$ = 0.0481 (25°±0.02°C))
Experimental medium = diluted sea water (Cl′ = 1.523 (25°±0.02°C); pH = 8.2 8.1; Δ = 1.53±0.01; $\varkappa$ = 0.0403 (25°±0.02°C))

Time in mins.	Chlorine ion in grs. per 100 c.c. (25°±0.02°C)	Δ	$\varkappa$ (25°±0.02°C)	0.4343 K′ $=\frac{1}{t}\log\frac{C_0-C_m}{C-C_m}$
0	1.905	1.91	0.0481	—
125	1.726	1.72	0.0442	0.0022
300	1.607	1.62	0.0422	0.0022
720	1.552	1.58	0.0408	0.0016
1445	1.535	1.55	0.0406	—
2025	1.529	1.53	0.0404	—

Oct. 22, 1930. Temp. = 14°±1°C.
Initial medium = normal sea water (Δ = 1.92±0.01; Cl′ = 1.91 (15°C); pH = 8.2)
Experimental medium = diluted sea water (Δ = 1.19±0.01; Cl′ = 1.17 (15°C); pH = 8.2)

0	1.91	1.90	—	—
60	1.76	1.78	—	0.0016
180	1.42	1.43	—	0.0026
390	1.31	1.34	—	0.0019
570	1.23	1.24	—	0.0019

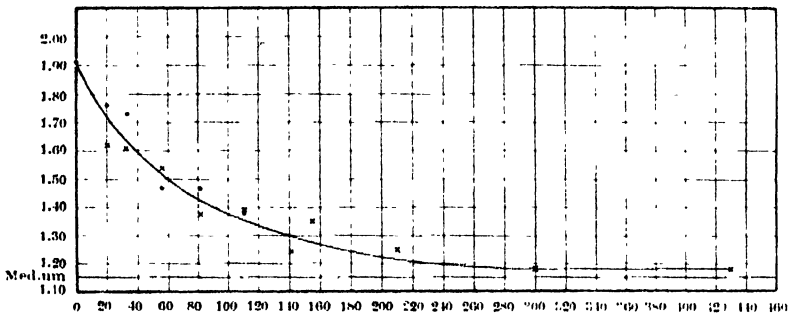
TABLE XXIX. — Change of the Body Weight of *Caudina* placed in diluted Sea Water (3 : 2).

Aug. 20, 1929. Medium temp. = 26°±1°C.

Time in minutes	Weight in grams
0	36.4
6	36.9
16	37.1
31	37.7

46	38.4
61	38.4
81	39.0
101	39.6
121	39.8
141	39.4
161	39.6
181	39.7
211	40.3
241	40.2
828	41.3

A



B

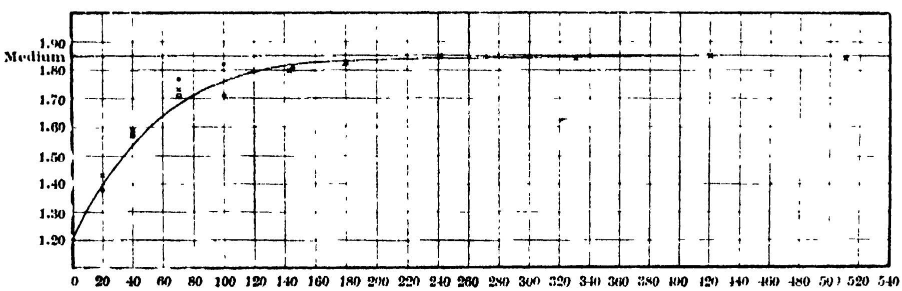


Fig. VII$_A$–VII$_B$. Change of concentration of body fluid of *Caudina*, its body, as a whole, being put in the medium (Table XXVI). Ordinate, contentration of Cl′ in grs. per 100 c.c. of body fluid; abcissa, time in mins.

A. Diluted medium (diluted sea water).
×··· small animals. ●··· large animals.

B. Concentrated medium (ordinary sea water)
×··· small sized animal. ●····middle sized animal.
□··· large sized animal.

The way of the osmotic equilibration may in this case also be expressed by the monomolecular equation to some extent, as in the preceding

experiments on the permeability of the body wall. The value of K′ for shrinking is also much greater than that for swelling, showing that consideration of the forces, chiefly such as an elasticity is also required. We however note that the concentration of the body fluid reaches that of the external medium much sooner than in the case of the permeation only across the body wall, which may be due to (1) periodical inhalation and exhalation of water through the anus, and (2) partly to swallowing of water through the mouth. There was no direct way to determine S/V. The data show that large animal which must have a large volume of the body fluid has a small K′.

It was often observed that the animal discharged the body fluid through the coeloanal canal, which made not only the conditions change but also sometimes the body weight decrease even when in diluted solution. So that in the present investigation, the animals which lost too much of their body fluid, were eliminated.

At any rate Table XXIX shows, swelling of the animal in the diluted medium was observed, sometimes its body being crammed to bursting with increased water in the body fluid indicating that the osmotic equilibration was produced in this case also mainly by the transport of water between the inside and the outside of the animal.

3. Permeability of the animal surface to Cl', SO_4'' $Na^{\cdot}$, $K^{\cdot}$, $Ca^{\cdot\cdot}$, and $Mg^{\cdot\cdot}$.

The following experiments were arranged so as to yield quantitative data on the permeation of $Na^{\cdot}$, $K^{\cdot}$, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, SO_4'', and Cl' into or out of the body fluid of *Caudinà*. According to the experiment (IV, B, 2), the composition of the body fluid of *Caudina* was very similar to that of the medium sea water. So that, as the experimental medium, the sea water of which the ratio of ions was changed but the osmotic pressure was not changed, was employed.

a. Method.

Experimental solutions were prepared by adding to sea water, measured proportions of salts solutions. The following Table demonstrates that 0.56 molar solution of a salt which molecule consists of two ions, has approximately the same osmotic pressure as the sea water.

The single salt solutions were, as had been expected, unphysiological even if isotonic, with animals. In isotonic NaCl solution *Caudina* could not live for more than 2 days; in isotonic KCl, $MgCl_2$ solutions the animal survived for twenty minutes. In KCl, the animal contracted to death discharging the body fluid; $MgCl_2$ solution acted as a narcotic; in $CaCl_2$ the

TABLE XXX. — A modified Table on the Composition of the Sea Water or the Body Fluid of *Caudina*, (see Table IV).

Concentration / Ion	grs. per L.	Gramions per L.	Gram-equivalents per L.	
Cl'	19.2	0.541	0.541	0.0599
SO_4''	2.8	0.029	0.058	
$Na^{\cdot}$	10.7	0.465	0.465	0.0602
$K^{\cdot}$	0.43	0.011	0.011	
$Ca^{\cdot\cdot}$	0.43	0.011	0.022	
$Mg^{\cdot\cdot}$	1.27	0.052	0.104	
Sum		**1.109**		

body wall became hard. Even in the equivalent mixture of these isotonic solutions, *Caudina* contracted to death soon, discharging the body fluid, but survival in this solution was possible if the mouth and anus were outside the solution; the same was the case in the mixture of NaCl and K_2SO_4. These circumstances lead me to prepare the following solution of a known composition which is slightly different from that of the sea water, but sufficiently different for the purpose.

Preparation of solutions.

0.055 × 4 M. KCl, 0.033 × 4 M. $CaCl_2$, 0.104 × 4 M. $MgCl_2$.6aq., 0.294 × 4 M. NaCl solutions were prepared; $CaCl_2$ and $MgCl_2$ solutions were standardized against the standard $AgNO_3$ solution. Then the equal volumes of these solutions were mixed.

The resulting solution contains:

		Sea water
Na	0.294 gramions/L.	0.465
$K^{\cdot}$	0.055 ,,	0.011
$Ca^{\cdot\cdot}$	0.033 ,,	0.011
$Mg^{\cdot\cdot}$	0.104 ,,	0.052
Cl'	0.623 { 0.055 (from KCl); 0.066 (,, $CaCl_2$); 0.208 (,, $MgCl_2$); 0.294 (,, NaCl)	0.541
SO''	0.000	0.029
Sum	**1.109**	**1.109**

In order to give the more physiological properties to the experimental solution, such as buffering action, the same pH as that of sea water *etc.*,

3 volumes of sea water were added to 4 volumes of this solution. The osmotic pressure of the resulting solution was determined by the freezing point method with an uncertainty of 1%, and regulated to that of the sea water which is in equilibrium with the animals, by addition of water or salt solution. This procedure was necessary as (1) the total concentration of salts in sea water, entirely different from the ratio of ions, is subject to the daily variations, (2) analysis of sea water was not complete when carried out by the micromethod.

Other methods and apparatuses, employed were the same as those of the experiments (IV; V).

The comparative data on the compositions of sea water, body fluid and experimental solutions are given in Table XXXI.

b. Results and discussion.

The observed compositions of the body fluid of *Caudina* after exposure of the animals to sea water modified so as to change the ratio of ions, but to have its osmotic pressure unchanged, has been tabulated in Tables XXXII, XXXIII and Figures $VIII_A$–$XIII_C$. As often stated, the volume of the experimental solution was so large that the concentration of each ion was not changed during the course of the experiment.

Since the number of times of the experiments was small and data for first several hours, in each experiment, are wanting owing to my expectation of smaller permeability, Tables XXXII, XXXIII are incomplete for studying kinetics of permeability. They will, however, answer the purpose of studying the permeability itself, or of showing the order of the magnitude of the velocity constant, K″, for the permeability to each ion. Here, it is also seen that K″ for the permeability of total body surface is much greater than that of the body wall.

Although I can say nothing about the so-called irreciprocal permeability (WHERTHEIMER 1923, 1925, 1926) and also the mutual influences of ions on their permeability, it seems that at least in the modified sea water, above mentioned, the order of their velocity of penetration is approximately as follows:

$$\begin{matrix} K^{\cdot} > Na^{\cdot} > Ca^{\cdot\cdot} > Mg^{\cdot\cdot}; \\ Cl' > SO_4'' \end{matrix} \quad \text{for permeability of the body wall.}$$

This order is the lyotropic and may be that of decreasing size of the hydrated ions (J. W. INGHAM '28; S. MATSUURA '30), and is parallel to that of their diffusion coefficients (LANDOLT BÖRNSTEIN-Physikalisch-Chemische Tabellen, 5 Auflage, 1, S. 246). This is also the order of permeability of cells of animals and plants (E. GELLHORN: Das Permeabilitätsproblem

TABLE XXXI. — Relation between the Sea Water (in Equilibrium with Animals) and the experimental Solutions.

Sea water and experimental solution	Δ	pH	Concentration in grams per 100 c.c.						Concentration in gramions per 100 c.c.						Sum as gramions per 100 c.c.	Sum of positive ions as gram-equi. per 100 c.c.	Sum of negative ions as gram-equi. per 100 c.c.
			Na˙	K˙	Ca˙˙	Mg˙˙	SO₄''	Cl'	Na˙	K˙	Ca˙˙	Mg˙˙	SO₄''	Cl'			
A. Sea water Nov. 30, 1930.	1.92	8.2	1.00	0.0408	0.0386	0.132	0.260	1.88	0.0435	0.00104	0.00096	0.00543	0.00271	0.0530	0.107	0.057*	0.058
Body fluid, in equilibrium with sea water. Nov. 30, 1930.	—	—	1.01	0.0442	0.0387	0.129	0.266	1.88	0.0440	0.00113	0.00097	0.00530	0.00277	0.0530	0.107	0.058*	0.059
Solution for permeability of body wall. Nov. 30, 1930.	1.93	8.2–8.0	0.84	0.139	0.086	0.208	0.112	2.07	0.0365	0.00356	0.00215	0.00855	0.00117	0.0584	0.110	0.062	0.061
Solution for permeability of total body surface. Nov. 30, 1930.	1.93	—	0.82	0.139	0.088	0.198	0.119	2.07	0.0357	0.00356	0.00220	0.00814	0.00124	0.0584	0.109	0.060*	0.061
B. Sea water Jan. 5, 1931.	1.92	8.2	1.00	0.0403	0.0386	0.136	0.276	1.91	0.0435	0.00103	0.00096	0.00559	0.00287	0.0539	0.108	0.058*	0.060
Solution for permeability of body wall. Jan. 5, 1931.	1.93	8.2–8.0	0.85	0.153	0.092	0.200	0.112	2.07	0.0370	0.00391	0.00230	0.00822	0.00117	0.0584	0.111	0.062	0.061

* The bases should be slightly greater than the equivalent of stable acid radicals but are not, due to the experimental errors.

TABLE XXXII. — The observed Compositions of the Body
Sea Water (Figs. $VIII_A$,

	Time in mins.	Concentration in the body fluid								
		as grs. per 100 c.c.						as gramions		
		$Na^{\cdot}$	$K^{\cdot}$	$Ca^{\cdot\cdot}$	$Mg^{\cdot\cdot}$	SO_4''	Cl'	$Na^{\cdot}$	$K^{\cdot}$	$Ca^{\cdot\cdot}$
A Nov. 30, 1930. 15° ±0.05°C.	Sea water in equilibrium with animals.	1.00	0.0408	0.0386	0.132	0.260	1.88	0.0435	0.00104	0.00096
	0 ×	1.01	0.0446	0.0387	0.131	0.266	1.88	0.0440	0.00114	0.00097
	0 ✱	1.01	0.0438	0.0387	0.126	0.266	1.88	0.0440	0.00112	0.00097
	290 □	0.91	0.118	0.069	0.172	0.201	2.00	0.0400	0.00302	0.00172
	772 ○	0.86	0.133	0.085	0.187	0.147	2.04	0.0374	0.00340	0.00212
	1580	0.83	0.141	0.085	0.203	0.137	2.07	0.0361	0.00361	0.00212
	3167 ◎	0.84	0.138	0.086	0.197	0.139	2.06	0.0365	0.00353	0.00215
	4209 △	0.83	0.143	0.093	0.204	0.135	2.07	0.0361	0.00366	0.00232
	7410	0.81	0.139	0.089	0.195	0.130	2.07	0.0352	0.00356	0.00222
	Medium	0.82	0.139	0.088	0.198	0.119	2.07	0.0357	0.00356	0.00220

× mix. from 4 animals. ✱ mix. from 2 animals. □ mix. from 3 animals.
○ mix. from 2 animals. ◎ mix. from 2 animals. △ mix. from 2 animals.

TABLE XXXIII. — The observed Compositions of the Body Fluids after
Anuses being out of the Medium (Figs. $VIII_B$, $VIII_C$,

	Time in mins.	Concentration in the body fluid								
		as grs. per 100 c.c.						as gramions		
		$Na^{\cdot}$	$K^{\cdot}$	$Ca^{\cdot\cdot}$	$Mg^{\cdot\cdot}$	SO_4''	Cl'	$Na^{\cdot}$	$K^{\cdot}$	$Ca^{\cdot\cdot}$
B Nov. 30, 1930. 15° ±0.05°C.	Sea water in equilibrium with animals.	1.00	0.0408	0.0386	0.132	0.260	1.88	0.0435	0.00104	0.00096
	0 *	1.01	0.0446	0.0387	0.131	0.266	1.88	0.0440	0.00114	0.00097
	0 §	1.01	0.0438	0.0387	0.126	0.266	1.88	0.0440	0.00112	0.00097
	602	0.92	0.125	0.057	0.156	0.188	1.99	0.0400	0.00320	0.00142
	1440	0.89	0.138	0.080	0.183	0.140	2.03	0.0387	0.00353	0.00200
	2880	0.86	0.143	0.088	0.195	0.128	2.06	0.0374	0.00366	0.00220
	4003	0.86	0.139	0.094	0.200	0.132	2.06	0.0374	0.00356	0.00235
	6915	0.84	0.144	0.086	0.210	0.131	2.07	0.0365	0.00368	0.00215
	Medium	0.84	0.139	0.086	0.208	0.112	2.07	0.0365	0.00356	0.00215

* mix. from 4 animals. § mix. from 2 animals.

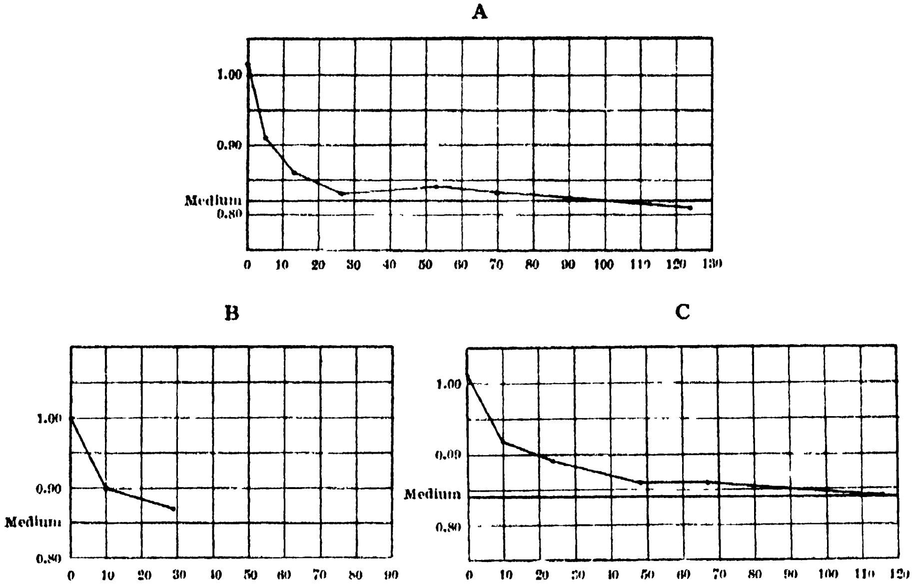
C Jan. 5, 1931. 15° ±0.05°C.	Sea water in equilibrium with animals.	1.00	0.0403	0.0386	0.136	0.276	1.91	0.0435	0.00103	0.00096
	564	0.90	0.135	0.069	0.177	0.195	2.02	0.0391	0.00345	0.00172
	1729	0.87	0.143	0.084	0.186	0.166	2.05	0.0378	0.00366	0.00210
	Medium	0.85	0.153	0.092	0.200	0.112	2.07	0.0370	0.00391	0.00230

Fluid after the Animals were placed in the modified
IX_A, X_A, XI_A, XII_A, $XIII_A$).

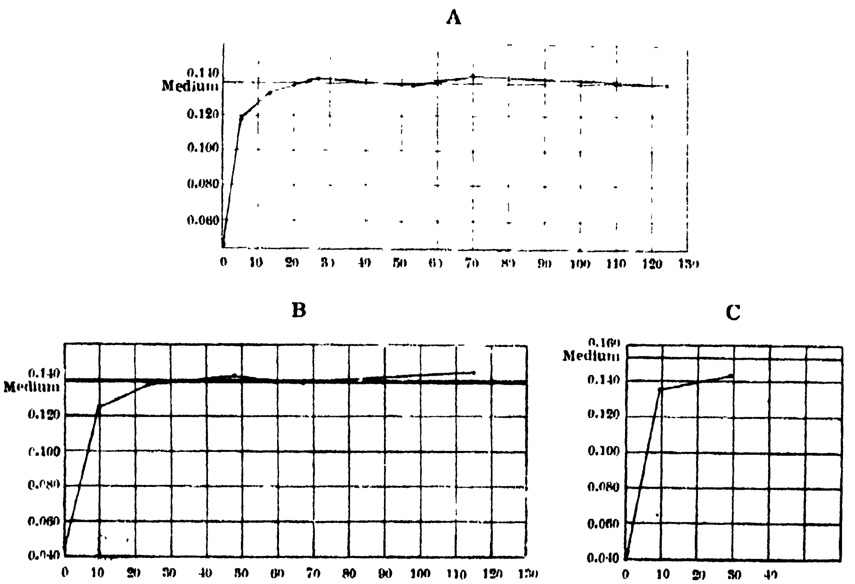
per 100 c.c.			Sum as gram-ions per 100 c.c.	Sum of positive ions as gram-equi. per 100 c.c.	Sum of negative ions as gram-equi. per 100 c.c.	$0.4343\ K'' = \frac{1}{t} \log \frac{C_{oi} - C_{mi}}{C_i - C_{mi}}$ 15°C					
Mg··	SO₄''	Cl'				Na·	K·	Ca··	Mg··	SO₄''	Cl'
0.00543	0.00271	0.0530	0.1066	0.0573	0.0584						
0.00539	0.00277	0.0530	0.1073	0.0590	0.0585						
0.00518	0.00277	0.0530	0.1070	0.0589	0.0585						
0.00707	0.00209	0.0564	0.1103	0.0606	0.0606	0.0011	0.0022	0.0014	0.0014	0.00097	0.0024
0.00769	0.00152	0.0575	0.1096	0.0604	0.0605	0.0009	0.0016	0.0016	0.0010	0.00093	0.0010
0.00835	0.00143	0.0584	0.1100	0.0607	0.0613					0.00058	
0.00810	0.00145	0.0581	0.1098	0.0605	0.0610					0.00027	
0.00839	0.00140	0.0584	0.1103	0.0612	0.0612					0.00029	
0.00802	0.00135	0.0584	0.1088	0.0592	0.0611					0.00015	
0.00814	0.00124	0.0584	0.1092	0.0599	0.0609						

Exposure of the Animals to modified Sea Water with their Mouths and
IX_B, IX_C, X_B, X_C, XI_B, XI_C, XII_B, XII_C, $XIII_B$, $XIII_C$).

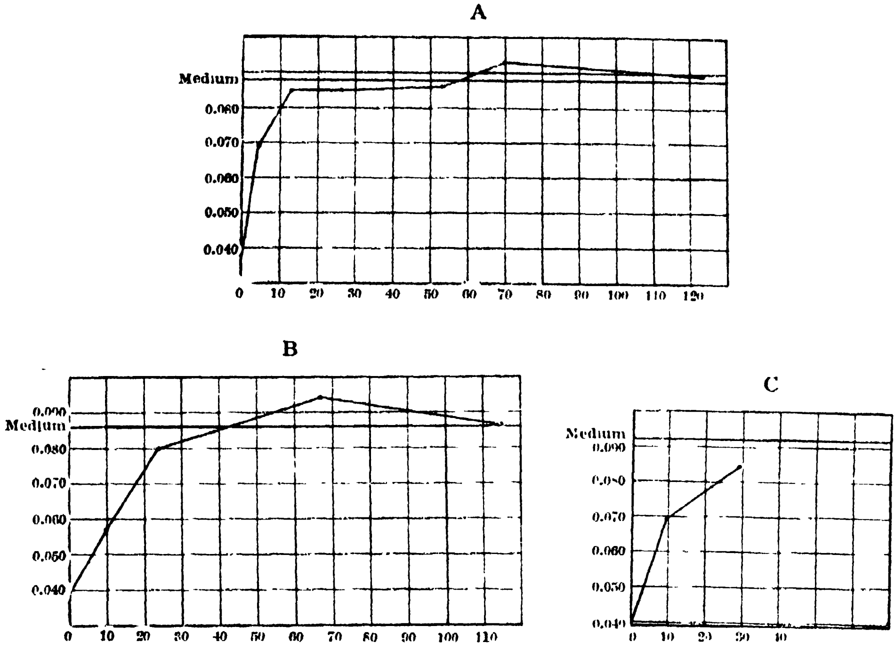
per 100 c.c.			Sum as gram-ions per 100 c.c.	Sum of positive ions as gram-equi. per 100 c.c.	Sum of negative ions as gram-equi. per 100 c.c.	$0.4343\ K'' = \frac{1}{t} \log \frac{C_{oi} - C_{mi}}{C_i - C_{mi}}$ 15°C					
Mg··	SO₄''	Cl'				Na·	K·	Ca··	Mg··	SO₄''	Cl'
0.00543	0.00271	0.0530	0.1066	0.0573	0.0584						
0.00539	0.00277	0.0530	0.1073	0.0590	0.0585						
0.00518	0.00277	0.0530	0.1070	0.0574	0.0585						
0.00641	0.00196	0.0561	0.1091	0.0589	0.0600	0.00054	0.0014	0.00035	0.00030	0.00051	0.00062
0.00753	0.00146	0.0573	0.1105	0.0613	0.0602	0.00037		0.00062	0.00035	0.00034	0.00047
0.00802	0.00133	0.0581	0.1107	0.0615	0.0608	0.00032			0.00027	0.00022	
0.00822	0.00137	0.0581	0.1110	0.0621	0.0608				0.00025	0.00013	
0.00863	0.00136	0.0584	0.1107	0.0617	0.0611						
0.00855	0.00117	0.0584	0.1103	0.0615	0.0607						
0.00559	0.00287	0.0539	0.1079	0.0576	0.0596						
0.00728	0.00203	0.0570	0.1106	0.0606	0.0611	0.00085	0.0014	0.00064	0.00079	0.00052	0.00090
0.00765	0.00173	0.0578	0.1107	0.0610	0.0613	0.00051	0.0006	0.00048	0.00038	0.00028	0.00052
0.00822	0.00117	0.0584	0.1110	0.0620	0.0607						



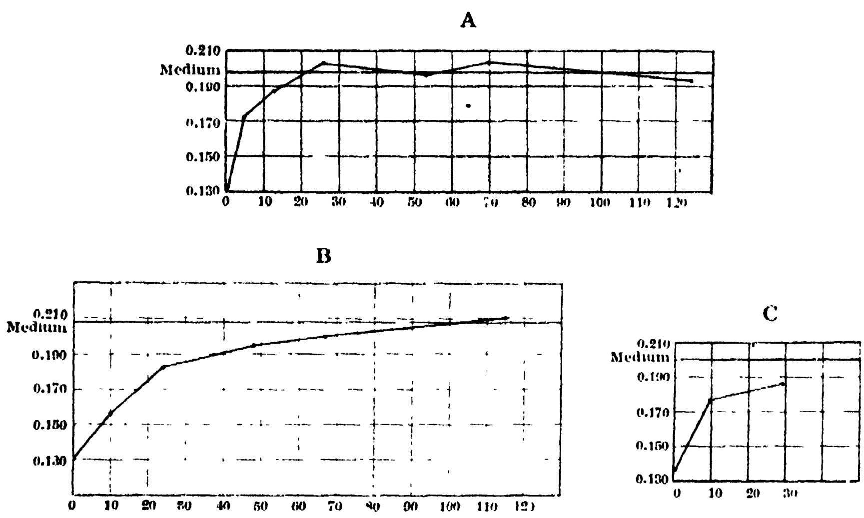
Figs. VIII$_A$, VIII$_B$, VIII$_C$: Na˙.



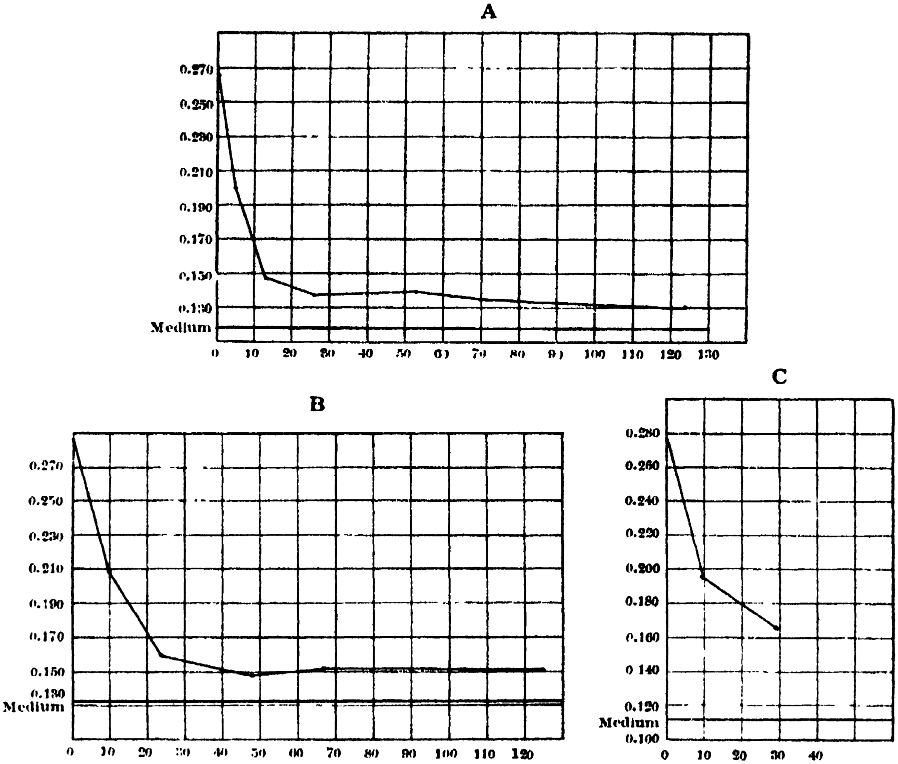
Figs. IX$_A$, IX$_B$, IX$_C$: K˙.



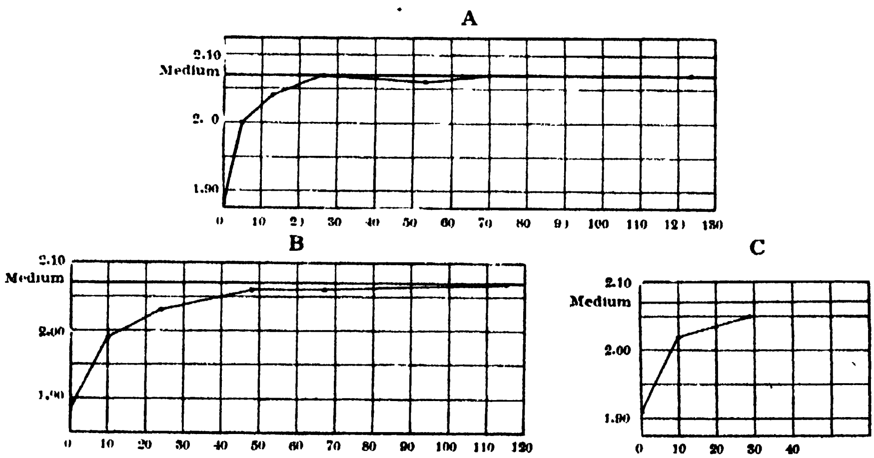
Figs. X_A, X_B, X_C: $Ca^{\cdot\cdot}$.



Figs. XI_A, XI_B, XI_C: $Mg^{\cdot\cdot}$.



Figs. XIIA, XIIB, XIIC: SO_4''.



Figs. XIIIA, XIIIB, XIIIC: Cl'.

Figs. VIIIA–XIIIC. The observed composition of the body fluid of *Caudina* in the modified sea water, (A) its body as a whole being put in the medium or (B, C) its mouth and anus being outside the medium (Tables XXXII, XXXIII). Ordinate, concentration of each ion in grs. per 100 c.c. of body fluid; abcissa, time in hrs.

(1929) — Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere 16. Band).

For the permeability of total body surface, the order seems to be as follows:

$K^{\cdot} > Ca^{\cdot\cdot}, Mg^{\cdot\cdot}, Na^{\cdot}$;

$Cl' > SO_4''$.

As to the order given above, I may state that, since discharging of the body fluid from the coeloanal canal as well as respiration in the respiratory trees came into question, as often stated, whether the discrepancy of the order shown between the permeability of the body wall and that of the total body depends on the experimental error or not, can not be decided and needs further investigation.

(According to S. MATSUURA, the velocity of permeation of a single electrolytc through a parchment paper is as follows:

$KCl > NH_4Cl > NaCl > LICl$; $BaCl_2 > CaCl_2 > MgCl_2$;

$KI = KBr > KCl > KNO_3 > K_2SO_4$).

VI. SUMMARY.

1. The body fluid, freed from corpuscles, of *Caudina chilensis* is almost of the same osmotic pressure (freezing point depression), specific conductance, specific gravity, and saline composition as those of the sea water in which it lives.
2. If the osmotic pressure (and specific gravity and specific conductance) of the sea water is changed by dilution or concentration within the limit of its survival, its body fluid rapidly attains almost the same osmotic pressure (and specific gravity and specific conductance) and this animal can not regulate the osmotic pressure of its body fluid to a value different from that of the medium.
3. The kinetics of the change of concentration of body fluid in diluted or concentrated sea water was discussed. Its velocity constant in the case of permeability of total body surface (the entire body being placed in the medium) was much larger than that in the case of permeability of body wall (mouth and anus being outside the experimental medium), which may be due to (1) periodical respiration of water in the respiratory trees which membrane is as thin as that of the alimentary tract, and (2) partly to swallowing of water through the mouth. It was seen that the velocity constant for shrinking of body was in both cases much greater than that for swelling, which depends probably on other forces such as elastic than

osmotic. For the swelling of the animal, previously shrunken in concentrated medium, in ordinary sea water in the case of permeability of body wall has the same velocity constant as that for shrinking. Therefore, irreciprocal permeability of the body wall to water seems not to exist.

It was proved that the osmotic equilibration occurs not only by diffusion of water but also by diffusion of salts in solution in the sea water, though it may be slight (a few per cent. of the former in the case of permeability of body wall).

4. The permeability of the animal surface, including or excluding mouth and anus, to each ion has been proved by the concentration change of each ion of the body fluid when the animal was placed in the sea water which was as modified in the ratio of $Na^{\cdot}$, $K^{\cdot}$, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, SO_4'', and Cl' but to maintain its original osmotic pressure and properties as physiological as possible; the kinetics of the phenomenon was discussed. Here it was also seen that the velocity constant of each ion for the permeability of total body surface is much greater than that of the body wall.

The rate of penetration of ions in this modified sea water was approximately as follows:

$K^{\cdot} > Na^{\cdot} > Ca^{\cdot\cdot} > Mg^{\cdot\cdot}$; $Cl' > SO_4''$ for permeability of body wall,

and $K^{\cdot} > Ca^{\cdot\cdot}, Mg^{\cdot\cdot}, Na^{\cdot}$; $Cl' > SO_4''$ for permeability of total surface.

5. The inhalation of the body fluid through the "coeloanal canals" does not appear to be the normal process with *Caudina*.

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EXPERIMENTAL STUDIES ON A JAPANESE PLANARIAN
I. FISSION AND DIFFERENTIAL SUSCEPTIBILITY.

By

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During a stay of some nine months as visiting professor at Tôhoku Imperial University, Sendai, Japan in 1930–31 an experimental study of the fresh water triclad known as *Planaria gonocephala* was undertaken to obtain material for comparison of this species with the American species studied. Various lines of experimental analysis which had been developed in the study of other species were followed with the Japanese species with particular reference to questions of gradients, dominance and polarity. The work included observations and experiments on fission, on various aspects of differential susceptibility, on reconstitution and head frequency in relation to length of piece and level of body and on the experimental modification of reconstitutional development.

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MATERIAL

The planarians were obtained from the Hirose river near Sendai, being found chiefly on the lower surfaces of the stones of the river bed. Collections were made both with use of meat as bait and by removing the animals from the stones. In keeping stocks of the animals in the laboratory some difficulty was encountered. Some stocks began to die within a few days after collection, others lived for some weeks and then

gradually died. The death rate was sometimes high in experimental lots while other similar lots showed no deaths. Lean beef, chicken liver, fish and earthworm were used as food and all were eaten readily, but the liver-fed and fish-fed stocks did not live well. However, since the food problem was considered only in so far as the attempt was made to provide a satisfactory food, nothing definite can be said concerning the possible factors in the deaths of these stocks. Lean beef was the most satisfactory food of those used.

It was also found that the tap water at the Institute was apparently toxic at times. Some stocks died within a few days after collection while others lived for several weeks. The water was stated to be in part the city water of Sendai, in part from a deep well. It was at times somewhat acid (pH 6.00 or even less) on account of excess CO_2 while at other times it was alkaline (pH 7.3). It was readily determined that the acidity of the water was not the toxic factor for the animals also died in water which had become alkaline by exposure to air, or was made alkaline by addition of $NaHCO_3$. A complete analysis of the water was not obtainable and the qualitative and quantitative salt content was not known. It was finally found that the animals lived well in various dilutions of a modified Ringer solution of the following composition: NaCl 6.5 g., KCl 0.14 g., $CaCl_2$ 0.12 g., distilled water 1000 cc. This solution was brought to pH 7.5–7.6 by addition of $NaHCO_3$. No deaths occurred in stocks kept for several months in dilutions of 1/20–1/50 of this solution.

In collections of the animals individuals ranging in size from 5–6 mm. to about 20 mm. were obtained. During the period of collection from October to May there was no indication of sexual reproduction and no sexual animals were found, but fission was of frequent occurrence except during the colder months. It is probable, therefore, that the small animals in the collected stocks were products of fission.

In color and general appearance the animals resemble *Planaria dorotocephala* but the head is less sharply pointed anteriorly and the cephalic lobes are shorter and blunter than in that species. On irritation or section the mucus secreted is greater in amount and much more dense and difficult to remove than in American species. It had been hoped that a study of physiological dominance in this species by means of grafts would be possible, but the large amount and the character of mucus secreted defeated all attempts at grafting. The grafts as well as the regions of the host body about the opening made for reception of the graft became covered with the dense mucus immediately after operation and since no

satisfactory method for its removal without inducing further secretion was found the tissues of graft and host apparently did not come into actual contact. Grafting was repeatedly attempted with animals in normal condition, with anesthetized animals, with animals made practically motionless by low temperature (operation on ice) and with animals in various concentrations of salt solutions, but without success. Even when the grafts remained in position for several hours or a day they were easily forced out by the contractions of the animals or often gradually emerged from the opening in the host body without active contraction on the part of the host.

FISSION UNDER NATURAL AND EXPERIMENTAL CONDITIONS

Reproduction by fission is characteristic of the species but under natural conditions rarely if ever occurs in animals less than 12-15 mm. in length. The body level at which fission usually takes place is 2–4 mm. posterior to the mouth but occasionally fission occurs at a distinctly more posterior level about half way between the usual level and the posterior end. In the larger individuals which have not recently undergone fission the postpharyngeal region is longer than the prepharyngeal. During the autumn most of the larger individuals collected showed evidences of recent fission in the short postpharyngeal region ending as if transversely cut or often in a regenerating posterior end. The postpharyngeal region grows much more rapidly than the anterior region and so attains the original length following fission with little or no increase in length of the whole individual.

Although no external morphological differentiation of a second individual is visible in the posterior region there can be no doubt that in this species, as in *P. dorotocephala*, this region is to some extent physiologically marked off from more anterior levels as another zooid and to a greater or less degree physiologically isolated from the dominant head of the animal (CHILD, 1910, 1911, 1924, pp. 151–160). Some of the physiological characteristics which distinguish the posterior zooid from more anterior regions will appear in the data presented below. In many of the larger animals the posterior region apparently represents two, or sometimes even three zooids. This is indicated by the fact that fission can readily be induced at one or two other fairly definite levels posterior to the usual level of fission and sometimes occurs at one of these levels in nature. At other levels of the body there is no indication of predetermined fission planes but in the absence of the head new zooids and fission planes will

develop and fission may occur in pieces from other levels. Various lines of evidence indicate that a posterior zooid is a region in which the reconstitution of a new individual has begun and is slowly progressing but does not attain a stage in which it becomes directly visible in morphological differentiation until after fission. For example, under slight stimulation, particularly in the absence of the head of the animal, the posterior zooid may show a motor reaction independent of the rest of the body; when physically isolated the head develops more rapidly and on a larger scale in relation to size of piece than in pieces from the middle region of the body and in *P. dorotocephala* the posterior zooid has a higher oxygen consumption than the middle region (HYMAN, 1923).

Certain seasonal differences in the lengths of animals and the occurrence of fission are of interest as indicating some of the factors concerned in determining whether, or at what length of body fission shall occur. As noted above, most of the longer animals collected during the autumn (October and November) had recently undergone fission and were 12–15 mm. in length and during the first few days in the laboratory further fissions occurred among those individuals 16–18 mm. in length which had developed posterior zooids since their last fission. Material collected from January to March showed that the animals were attaining greater length (20–25 mm.) during this period than in the autumn but these animals showed no evidences of recent fission and under laboratory conditions in water at a higher temperature than in nature no fissions were observed. Material collected during April was similar in character, but early in May fissions began to occur again in nature and somewhat later in the month almost all of the larger individuals showed the short blunt and regenerating posterior ends and the small animals which develop from the posterior zooids were much more numerous than earlier. Collections could not be continued during the summer but since no indication of development of sex organs was observed in the spring material and since the autumn material showed numerous fissions, it seems probable that fission continues during the summer without a period of sexual reproduction. This is the case in *P. dorotocephala* in the localities from which collections have been made and the absence of sexual reproduction has also been noted for other species in certain localities in the United States.

A few experiments on fission were made, in part by the writer, in part by students. It has long been known that in planarian species which undergo fission removal of the head will frequently induce its occurrence, even in animals of small size which very rarely or never undergo fission

in nature. When the head is removed fission does not usually occur at once but only after several days when the new head has developed sufficiently so that the animal becomes active again. Apparently at this stage the dominance of the new head over the posterior zooid is less complete than that of the original head and as the animals begin to move about an independent reaction of the posterior zooid occurs more readily than in the intact animal. If fission does not occur within a week after removal of the head it rarely occurs later except after feeding and further growth in length or a second removal of the head. Within a week after removal of the head the dominance of the new head is evidently sufficient to prevent fission.

Table I gives data on the occurrence of fission following removal of the head in large and small animals in early autumn and in spring. The table shows that in all lots fission occurs more frequently in large than in small animals following removal of the head, also that it occurs more frequently in the autumn than in the spring material and finally that in the spring material a second removal of the head about one week after the first removal induces a few more fissions in the larger animals but has no effect on the small animals. Both in the large and small animals the total percentages of fission after the second removal of the head are less in the April material than in the autumn material after the first removal of the head, but in the May material the total percentage of fission after the second removal of the head approaches that of the autumn material. In the April and May material some deaths occurred but only in the May material is the number of deaths sufficient to make the results of uncertain comparative value.

TABLE I.

Occurrence of fission after removal of head in large and small animals at different seasons.

Season	Number of animals	Length in mm.	After first removal of head		After second removal of head	
			Dead	Number of fissions as per cents of living	Total dead	Total number of fissions as per cents of living
October	40	18–20	0	65		
	40	8–10	0	35		
April	40	20–22	2	10	5	45
	50	8–10	3	25.5	3	25.5
May	20	18–20	6	42.8	6	57

As already noted above fissions occurred among the larger animals of the autumn stocks without removal of the heads, but somewhat less frequently than when the heads were removed. In the winter and early spring material the large animals did not undergo fission unless the heads were removed although they were considerably longer than the autumn animals. In the smaller animals (under 12 mm.) of the autumn material fission was very rare unless the heads were removed and in the winter and spring material it was nerver observed except after removal of the head.

Both the observations on material collected at different seasons and the effects of removal of the head show that animals which have been living at low temperature do not undergo fission as readily as those which have lived at higher temperature, even though they attain a much greater length than the latter. There is considerable evidence for several species to indicate that rate of growth is an important factor in determining the length attained before fission. The more rapidly growth in length occurs the less the length at which fission occurs. With slow growth in the laboratory *P. dorotocephala* may attain without fission twice the length that it reaches in nature at the same temperature but with a much higher growth rate and in *P. maculata* a greater length is attained without fission when growth is slow than when it is rapid.

It may be suggested as a possible basis for these differences that when growth in length is rapid the differentiation and functional development of the nervous system do not keep pace with it, consequently a sufficient degree of physiological isolation of the posterior region to permit fission occurs at a shorter length than when growth is slow. In the case of the animals living at low temperature it is possible that a lower level of motor activity may decrease the frequency of fission while the animals are in the cold water, but that this is not the chief factor is shown by the fact that when these low temperature animals are brought into warmer water in the laboratory they do not undergo fission.

In animals like *Planaria* which continue to grow in length throughout life the longitudinal nerve cords must undergo continuous development corresponding to the increase in length of the body and the distance or range over which the dominance of the head is effective must be limited by the development of the nerve cords in the posterior region in which growth is most rapid, particularly in the larger animals. With rapid growth in length development in the posterior region may occur more or less as it does in a physically isolated piece and the result is a new zooid. But this zooid is prevented from developing beyond very early stages until

fission occurs and the range of dominance of its anterior region remains very short so that with further growth in length a second physiological isolation may occur with demarcation of another zooid, and if fission does not occur a fourth zooid may develop. All of these posterior zooids are short because the head region and the nervous system are but little developed, that is, the nervous system has undergone but little reorganization in the direction of a new individual, consequently the range of dominance and the lengths of these are short. The anterior zooid, on the other hand, with fully developed head and with little reorganization of the longitudinal cords in the later stages of growth since it undergoes only a very slow increase in length, attains a much greater length than the posterior zooids. This difference in range of dominance in relation to degree of development of the head, that is, the nervous system, becomes visible in the rhabdocoel *Stenostomum* in the different lengths which different zooids of the chain attain before their posterior regions become marked off as new zooids (CHILD, 1929, pp. 43, 44).

As regards development of new zooids in the posterior region and occurrence of fission the Japanese species and *P. dorotocephala* are very similar. Such species cannot become sexually mature as long as nutrition and rate of growth lead to the development of posterior zooids and the occurrence of fission. Even if testes and ovaries develop the repeated reorganization of the postpharyngeal region associated with fission prevents the development of the terminal portions of the reproductive system. But apparently this repeated reorganization, together with periods of scarcity of food, keeps the animals in a physiological condition or stage in which development of sex organs does not occur, that is, the tissues do not attain the physiological age at which these organs appear (CASTLE, 1928; CHILD, 1913 b, 1914 b, 1915, chaps. V–VII). In the laboratory under conditions which permit only slow growth *P. dorotocephala* may become sexually mature after several months without fission. Probably the Japanese species can be brought to sexual maturity in the same way and in some localities where natural conditions favor slow growth and fission occurs rarely or not at all sexual maturity may appear regularly as a stage of the life cycle.

DIFFERENTIAL SUSCEPTIBILITY: INTRODUCTORY

The facts of differential susceptibility and their physiological significance have been discussed elsewhere (CHILD, 1913 a, 1924, pp. 76–86, 104–110,

1928, 1930). Here only a general statement of the present theory of differential susceptibility and the more important facts concerning *P. gonocephala* as compared with the American species are given.

First, the differences in susceptibility of different regions or organs of an individual to physical and chemical agents may be non-specific gradients, that is, in the same direction though perhaps different in steepness or slope for a certain range of concentration or intensity of action of different agents. On the other hand, these differences in susceptibility may be specific for particular agents and may appear as gradients differing in direction with different agents or as more or less sharply localized specific susceptibilities of particular organs or tissues.

Second, non-specific susceptibility gradients indicated by the gradual progress of cytolysis or other death changes or by different degrees of retardation or inhibition of growth, development or motor activity are very generally characteristic of physiological axes, at least during the earlier stages of development and often in the simpler organisms or in particular organs throughout life.

Third, non-specific susceptibility gradients, that is, those which show the same direction with different agents which produce their toxic or lethal effects in different ways, must depend on quantitative rather than on qualitative or specific differences in physiological condition in the axis or direction concerned.

Fourth, the evidence at hand indicates that these quantitative differences in condition are usually if not always associated with and indicated by differences in rate of respiratory metabolism. To a certain range of highly toxic or gradually lethal concentrations or intensities of action of agents the susceptibilities vary directly with the quantitative differences in physiological condition. To a certain range of low concentrations or intensities of action of the same agents the susceptibilities vary in all cases investigated inversely with the quantitative differences in physiological condition. In other words, to the high range of concentrations and intensities of action the most active regions are most susceptible, while to the low range these regions are least susceptible, acquire tolerance or acclimate most rapidly and recover most rapidly after temporary exposure up to a certain limit of duration.

These general relations between the rate of change in the system and susceptibility to external disturbance hold, not only for living protoplasms, but for other physicochemical systems undergoing dynamic equilibration. The higher the rate of change in the system the more rapidly is the

system disrupted or essentially altered by extreme irreversible external disturbance and the less susceptible it is to slight, rapidly reversible disturbance. The rate of change, the degree of activity of the system is then an essential factor in the effect of the external disturbance. In the case of a toxic action of an agent on living protoplasm above the limit of tolerance the most active region will reach the point where normal activity cannot continue sooner than other less active regions because its own activity as well as the concentration of the agent is a factor in bringing it to this point. To an action within the limit of tolerance the most active region will be least susceptible because it will most rapidly dispose of the agent by oxidation, reduction or otherwise and will most rapidly remove or reverse its effects. For example a planarian or a region of the planarian body with a high rate of metabolism dies more rapidly than other individuals or regions with a lower rate in alcohol at a concentration of four or five per cent, but in a concentration of one to one and a half per cent this individual or region may live indefinitely while the animal or region with lower metabolism may gradually die in the same concentration. Again, as will appear below, when isolated pieces of *Planaria* undergo reconstitution in KCN m/300000 or m/400000 the development of the head is less inhibited than that of the posterior end but in KCN m/10000 or higher concentration the head is the most susceptible region and disintegrates first (see p. 334).

With the progress of differentiation, particulary in the higher animals, specific susceptibilities often appear in particular organs or tissues, that is, one organ may be highly susceptible to a certain agent, another organ to another. These specific susceptibilities are evidently dependent on the qualitative or specific differences in constitution of different organs or tissues and in action of different agents. But a differentiated organ which differs specifically from other organs in its susceptibility may show quantitative non-specific differences in susceptibility along its axis or axes, that is, a tissue which has undergone differentiation may still show the quantitative, non-specific differences in susceptibility in its different regions.

DIFFERENTIAL SUSCEPTIBILITY AS INDICATED BY DIFFERENTIAL DEATH

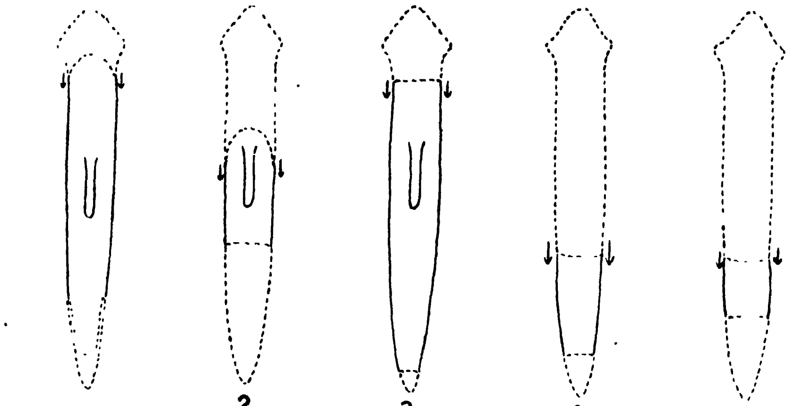
Observation on the differential susceptibility of the Japanese species to killing concentrations shows that it is very similar to *P. dorotocephala* and *P. maculata*, consequently the important facts are presented in brief general form without tables or graphs.

In the planarian body differential susceptibility is in part non-specific or quantitative, that is, the differences shown by different agents are in the same direction with respect to the axes, but certain more or less specific susceptibilities also appear. The differential susceptibility of *P. dorotocephala* to a large number of agents has been determined. These agents include weak and strong acids and bases, cyanides and various other electrolytes, various anesthetics, caffein, nicotin, vital dyes, distilled water, hypertonic solutions, ultraviolet radiation, x-rays, radium, extremes of temperature, lack of oxygen. The number of agents used with the Japanese species is much less, but sufficient to show the general similarity to *P. dorotocephala.* It includes the following: KCN in various concentrations from m/1000 to m/10000 in alkaline, neutral and acid solutions, ethyl alcohol 3–4 per cent, ethyl ether 1–1.5 per cent, chloretone 1/20–1/5 saturation, NH_4OH (1/50000–1/100000 ammonia water), distilled water. The ranges of concentration given are those found by preliminary experiment to be above the limit of tolerance and below that of instantaneous killing.

Because of the high susceptibility of the gut in recently fed animals the differences in susceptibility of the body surface and body wall appear more clearly in animals which have been without food for at least several days. In the recently fed animals agents which penetrate readily may affect the gut earlier than the body wall to such an extent that portions of the cytolyzing gut break through the body wall. Moreover, there is evidence that the actively digesting gut affects the physiological condition of the dorsal body wall so that it cytolyzes earlier in localized areas directly dorsal to parts of the gut. In animals kept without food for a week or two the gut is much less susceptible as compared with the body wall and with longer starvation the susceptibility decreases still further.

In general disintegration or other death changes in the anterior zooid begin at the head and progress posteriorly to the level at which fission usually occurs. The susceptibility of the posterior zooid region as compared with that of the anterior zooid differs with size of animal and with various other conditions. For example, in animals 15–20 mm. in length in KCN m/1000 or m/2000 with pH above 9 the posterior tip of the body shows about the same susceptibility as the head and the whole posterior zooid region disintegrates before the posterior region of the anterior zooid (Figs. 1 and 2). In the same concentrations of KCN but with pH near neutrality on either side death occurs much less rapidly than in the strongly alkaline solutions, the posterior tip is somewhat less susceptible as compared with

the head and the posterior zooid region or the anterior half of it is the last part of the body to die and disintegrate (Figs. 3–5). With the slower progress of death the posterior zooid region of larger animals often



1 2 3 4 5

Figs 1–5.—Differential susceptibility as indicated by death and disintegration. The parts of the bodies drawn in broken lines are those in which surface and body wall have disintegrated. Arrows indicate direction of progress of disintegration. Figs. 1 and 2, disintegration in strongly alkaline or acid KCN and various other agents; the posterior region of the first zooid is least susceptible and at a given level the median region is less susceptible than the lateral margin. Figs. 3–5, disintegration in KCN near neutrality and in various non-irritating agents. Posterior zooid region or anterior half of it is the least susceptible region; margins are no more susceptible than median region.

shows a distinct division into two parts as regards susceptibility, the posterior part being the more susceptible (Fig. 5). This suggests the presence of two zooids in this region and the experiments on fission show that two zooids are often present (p. 315). In KCN solutions with pH below 5 the acidity becomes the factor which kills, the susceptibility is higher than in less strongly acid solutions, and the relative susceptibility of the posterior zooid region approaches that in the strongly alkaline solutions. In the other agents used some variation in the relative susceptibility of the posterior zooid region also appears. It may be as susceptible as the region just posterior to the head or it may be the least susceptible region of the body according to concentration, pH and perhaps other factors.

In *P. dorotocephala* the posterior zooid region has a higher oxygen consumption than the posterior half of the anterior zooid (Hyman, 1923)

but whether or not this is the case in *P. gonocephala* is not yet known. In general the relative susceptibility of the posterior zooid region to killing concentrations appears to be somewhat lower in *P. gonocephala* than in *P. dorotocephala.* In both species the susceptibility of the posterior zooid region is increased or decreased more than that of other parts of the body by change in pH and in the lower killing concentrations of various agents its relative susceptibility decreases, that is, its survival time, as compared with that of the anterior zooid, increases, probably because it has a slightly higher limit of tolerance and oxidizes, reduces or otherwise disposes of the toxic agent somewhat more rapidly than the posterior parts of the anterior zooid. These variations in the relative susceptibility of the posterior zooid region show beyond question that it differs at least quantitatively in physiological condition from the posterior parts of the anterior zooid. It is a region in which the beginnings of reorganization into one or more new individuals are slowly taking place and it is probably to be regarded as slightly younger physiologically than adjoining regions and its higher oxygen consumption in *P. dorotocephala* is in line with this suggestion.

The lateral marginal regions of the body show certain features of interest as regards susceptibility. In the more strongly alkaline and more strongly acid agents and in general in agents or concentrations which induce marked irritation or stimulation in the earlier stages of their action the lateral margins die and disintegrate earlier than median regions of the same body level (Figs. 1, 2). In slightly alkaline, neutral, or slightly acid solutions and with agents which do not irritate death and disintegration of marginal and median regions of a given body level occur almost at the same time, the median often slightly in advance of the lateral region (Figs. 3–5). These apparently more or less specific differences in susceptibility seem to depend, at least in large part, on the presence of large numbers of mucus gland cells in the marginal region, but sensory cells of this region may also contribute to the high susceptibility. The mucus cells are stimulated to secretory activity by a pH above and below a certain range and by various other irritating agents and this stimulation evidently increases the susceptibility of the margins. With a pH near neutrality and in non-irritating agents or concentrations these cells are not stimulated and under these conditions the margins are no more, or even less susceptible than the median region. The susceptibility of the dorsal as compared with the ventral body wall shows somewhat similar variation with pH and other irritation and this also apparently results

from some degree of specific difference between dorsal and ventral regions.

As regards the parenchyma and internal organs it is impossible to determine directly by means of differential killing whether or what differences in susceptibility are present or what the significance of observed differences may be because it is impossible with most agents to be certain whether exposure of the internal organs and tissues has been uniform in different agents. Certain internal regions may cytolyze earlier than others because earlier destruction or alteration of the body wall in those regions permits the agent to reach them earlier or in higher concentration. For example, if cytolysis of the gut progresses from the anterior end in the posterior direction following cytolysis of the body wall it does not necessarily mean that this course of cytolysis represents the real gut gradient. In fact, it probably does not for with agents which penetrate readily to the interior of the body the pharyngeal region and the middle region of the gut appear usually to be the most susceptible, though the susceptibility may vary with amount of food present and stage of digestion. The early swelling and cytolysis of gut cells with local cytolysis of the body wall dorsal to gut branches apparently occurs to a greater extent in fat-soluble agents, e. g., ether, chloretone and various other anesthetics, particularly in the higher concentrations. This is what might be expected for the gut cells are storage cells as well as digestive cells and in well fed animals accumulate fat (WILLIER, HYMAN and RIFENBURGH, 1925). In susceptibility tests made immediately or a few hours after feeding such breaking through of the gut cells may occur more or less all over the body. In animals which have been starved for several weeks the gut is relatively much less susceptible and at a given body level may remain intact or almost intact for some time after disintegration of the body wall. The susceptibility of the pharynx seems to be to some extent specific. In KCN it is little if any more susceptible than the regions about it, but in various anesthetics and various other agents it is affected very early and may break through the dorsal body wall while that is otherwise intact and may even separate from the body. It seems probable that this specificity in susceptibility may be associated with the high neuromuscular development of this organ.

From these various facts it appears that the longitudinal polar gradient of the body surface and body wall of *P. gonocephala*, like that of *P. dorotocephala* and *P. maculata* is non-specific, that is, in the same direction for all agents used. The differences in susceptibility in the three species, so far as observed, are differences of degree rather than qualitative or specific differences. As will appear below, the differences in suscepti-

bility to killing agents along the polar axis correspond to differences in rate of growth and of susceptibility of the growing tissue in isolated pieces undergoing reconstitution. In the light of all the evidence it cannot be doubted that these differences in susceptibility have a real physiological significance and that they are the expression of a quantitative differential or gradient in physiological condition along the axis. The high susceptibility of the posterior tip of the body is probably associated with its activity as a growing region, but its function as a region of attachment may also be concerned. The appearance of a certain degree of specificity for certain agents in the differences in susceptibility between median and lateral and between dorsal and ventral is in accord with the morphological evidence in indicating the existence of specific differentiations in different parts of these axes.

In a recent paper J. W. WILSON (1931) has attempted to show by substitution of diluted Ringer solution for water that KCN merely brings about rupture of the margin of the body in *Planaria* through swelling of the granules in the ducts of the marginal mucus glands and that death and disintegration result from the exposure of the internal tissues to the hypotonic water. In the Ringer solution of KCN cytolysis does not occur as it does in water. When we recall that KCN is a powerful inhibitor of most physiological oxidations and that in a concentration of m/2000 in water it decreases oxygen consumption in *Planaria* 80 to 90 per cent (HYMAN, 1919 a it is rather difficult to believe that water and not KCN is the cause of death and disintegration of *Planaria* in an aqueous solution of KCN in concentration of m/1000. WILSON believes that neither alkalinity nor effectiveness of the KCN are altered in his Ringer-KCN solution, but he does not give the pH of the solution nor state whether it is buffered. In his attempt to show that there is no anteroposterior gradient in *Planaria*, WILSON fails to mention the fact that in neutral cyanide and in various other agents disintegration of the lateral margins does not occur much or any earlier than that of the median region and may even occur later than median disintegration. In such agents disintegration progresses directly posteriorly from the head over the length of the anterior zooid and usually also over the second zooid, as described above (p. 323. Figs. 3, 4; see also BUCHANAN, 1930, CHILD, 1930) so that the last part to disintegrate is not the pharyngeal region but a level considerably posterior to it. Such a course of distintegration cannot possibly be interpreted as progressing from the lateral margins to the median region. In many other fresh water animals which do not possess the marginal structures found in *Planaria*

cytolysis occurs in aqueous solutions of KCN with apicobasal or antero-posterior gradient over at least a considerable part of the body. This is the case, for example, in *Paramecium* and *Hydra* (CHILD and DEVINEY, 1925; CHILD and HYMAN, 1919), *Stenostomum* (CHILD, 1924, p. 81, Fig. 42) and various oligochetes (HYMAN, 1916). Moreover, similar gradients have been observed with KCN as agent in eggs, blastulae, planulae, larvae and later stages of various marine forms and these forms not only lack any such structural differentiation as the margins of the planarian body, but they live in a balanced salt solution, that is, in sea water. More conclusive evidence than WILSON has given is necessary to show that the action of KCN on *Planaria* differs from that on all these forms.

SUSCEPTIBILITY TO DISTILLED WATER

The Japanese species is much less susceptible to distilled water than *P. dorotocephala* and in this respect resembles *P. maculata* (CHILD, 1930), but its susceptibility, like that of the other species depends on the number and size of individuals in relation to the volume of distilled water (BUCHANAN, 1930, CHILD, 1930). Apparently the animals give off sufficient salts to condition the water and if the volume of water is not too large or the volume of animals too small and if the water is not changed, they may live indefinitely. The ability of *P. gonocephala* to live in distilled water is shown by the following data.

I A. 5 animals 15 mm. in length in 500 cc. distilled water with I B (see below). Distilled water changed on 4th., 5th., 13th. and 15th. days. After 22 days three individuals alive, two intact, one without head but healing has occurred. At this time two individuals, one intact, one headless, were cut into six pieces each and returned to tap water in which they underwent reconstitution, all except one piece giving rise to normal animals. The other individual remained in 500 cc. distilled water which was changed every three or four days. After 42 days the head had disintegrated but the anterior end had healed. After 50 days it was still in the same condition, but after a further interval of three weeks without observation or change of water was dead.

I B. 5 animals 7–8 mm. in length, together with I A in 500 cc. distilled water. Water changed as in I A. After 5 days two alive; after 13 days all dead.

II A. 5 animals 16–18 mm. in length in 50 cc. distilled water. Water changed on 3rd., 4th., 12th., 17th. days. After 20 days all intact. Observation not continued.

II B. 5 animals 7–8 mm. in length in 50 cc. distilled water. Water changed as in II A. After 20 days four intact. Observation not continued.

Although experiments II A and II B were not carried to conclusion it is evident that these lots in 50 cc. of distilled water are less susceptible than those in 500 cc. in spite of the repeated change of water. It is also evident that small, physiologically young animals (I B, II B) are more susceptible to distilled water than the large old individuals. By way of comparison with *P. gonocephala* it may be noted that when a lot of ten individuals of *P. dorotocephala* 18–20 mm. in length is placed in 500 cc. distilled water all die within 24–48 hours without change of water and small animals 7–8 mm. in length die in half or less than half this time. Of ten animals 18–20 mm. in length in 50 cc. distilled water some die within 4–5 days and some may live longer, perhaps indefinitely if the water is not changed and if disintegrating individuals do not foul it. If the water is changed every third or fourth day the animals die within a week or ten days. Ten animals 7–8 mm. in length in 50 cc. distilled water usually all die in 2–3 days without change of water, but often the earlier death and disintegration of some will prolong the lives of others, presumably by setting free small amounts of salts.

These are characteristic results at temperatures near 20°C. The survival times differ somewhat with nutritive condition and with pH of the distilled water but the relations between susceptibility and size and number of animals and volume of water remain the same. It is evident that *P. gonocephala* is much less susceptible to distilled water than *P. dorotocephala*. Some individuals of the Japanese species are able to live for long periods, perhaps indefinitely in distilled water, even with frequent change of water, while under such conditions *P. dorotocephala* always dies within a few days at most. A few experiments with *P. maculata* (CHILD, 1930) show that this species is also less susceptible to distilled water than *P. dorotocephala*, but apparently somewhat more susceptible than the Japanese species.

These differences in susceptibility to distilled water appear to be associated with differences in the salt content of the waters in which the animals occur. Up to the present time *P. dorotocephala* has been found only in the waters of springs with a relatively high calcium content while *P. maculata* and the Japanese *P. gonocephala* are found in streams and ponds in which the calcium content may be low. Both of these species, however, will live in waters with a wide range of salt content while *P. dorotocephala* is apparently adjusted to a higher ratio of calcium to potassium (MURRAY, 1928).

The disintegration gradient of *P. gonocephala* in distilled water is essentially similar to that in slightly alkaline, neutral or slightly acid KCN and various other solutions near neutrality (Figs. 3–5; see p. 322 above) and to that of *P. dorotocephala* and *P. maculata* in distilled water (BUCHANAN, 1930, p. 311; CHILD, 1930, p. 118), that is, the whole posterior zooid region except the extreme posterior tip of the body (Fig. 4), or in some individuals only the anterior half of this region (Fig. 5), is less susceptible than levels immediately anterior to it. Disintegration begins at the head and usually somewhat later at the posterior tip of the body and progresses posteriorly from the head to the posterior tip or to the middle of the posterior zooid region. Here, as in KCN near neutrality, when the posterior region consists of a single well defined zooid the disintegration progresses posteriorly to the level of the posterior tip (Fig. 4) but when two zooids are present in the posterior region the more posterior of the two is the more susceptible (Fig. 5). The distilled water used in these experiments is slightly acid or neutral and of course cannot be made alkaline without addition of salts, that is, it can be used only as an approximately neutral agent.

SUSCEPTIBILITY IN RELATION TO SIZE OF INDIVIDUAL

Small individuals of *P. dorotocephala* and *P. maculata* have a higher rate of respiration than large (HYMAN, 1919 b, ROBBINS and CHILD, 1920, confirmed by others), that is, the small animals, whether they develop from eggs, fission pieces or pieces experimentally isolated, are physiologically younger than the large. Undoubtedly this is also true for *P. gonocephala*. In *P. gonocephala*, as in the other species, the smaller individuals are more susceptible than the larger to a certain range of killing concentrations. In large animals which have been heavily fed for some time the susceptibility of the gut to fat-soluble agents may be so great that it breaks through the body wall and brings about disintegration as early as, or earlier than in the small animals. This high susceptibility of the gut is apparently due to its high fat content as well as its functional condition, for after two weeks or more of starvation it has usually disappeared and the large animals are then less susceptible than the small, even in the fat-soluble agents.

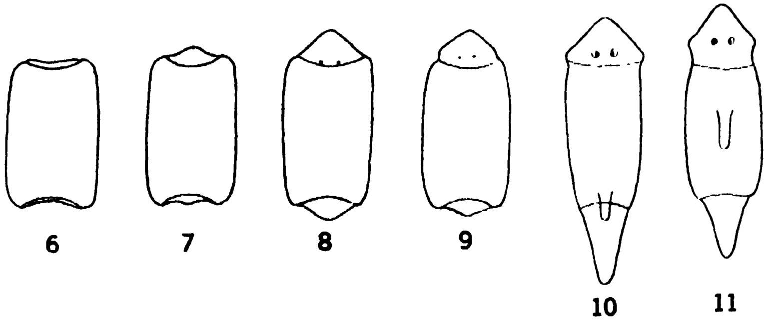
In a certain range of low concentrations the smaller animals are less susceptible and live longer than the larger. For example, in chloretone 1/5 saturation individuals 6–7 mm. in length die earlier than individuals

16–18 mm. but in chloretone 1/20 saturation or less the small individuals in general live longer than the large. In concentrations of HCl below a certain pH, which depends on size, physiological condition of animals and temperature, but is somewhere about pH 5, the smaller animals are more susceptible than the larger. With increase in pH we find that at a certain pH near pH 5 the small animals become less susceptible and live longer than the large or perhaps indefinitely in the acid solution. This reversal of the susceptibility difference in large and small animals has not been investigated further in *P. gonocephala* but in *P. dorotocephala* it has been observed with decrease in concentration or intensity in acids, ethyl alcohol, ethyl ether, to a slight degree in very low concentrations of KCN and in low temperature (mostly unpublished data), and incidental evidences of it have appeared in the use of other agents, but have not been further investigated.

DIFFERENTIAL SUSCEPTIBILITY IN RECONSTITUTION IN LOW CONCENTRATION OF KCN

The reconstitutional development of heads in relation to length of piece and level of body and the experimental modification of head frequency and other features of reconstitution will be considered in another paper, but attention must be briefly called at this time to certain points in reconstitution of pieces in water as a basis for comparison with the results of experiment.

Pieces undergoing reconstitution in water show in the earlier stages a higher rate of growth of new tissue at the anterior than at the posterior end of the piece. At temperatures near 20°C. this difference is usually clearly distinguishable within forty-eight hours after section (Fig. 6) and becomes more distinct after three days (Fig. 7). As the new tissue increases in amount it also becomes evident that the rate of growth of both anterior and posterior new tissue differs according to the level of the body from which the piece was taken. In general the growth rate decreases from the anterior region posteriorly to the level of fission and in pieces from the posterior zooid region it increases again. Figure 8 shows the usual condition 5–6 days after section in a 1/6 piece from a level just posterior to the head and Figure 9 the condition at the same time in a similar piece from the posterior part of the anterior zooid, i. e., in or near the oral region. It will be observed that not only is the scale of organization of the head larger but development of the head, as indicated by the eyes, is farther advanced in Figure 8 than in Figure 9. At the posterior ends



Figs. 6-11.—Growth of anterior and posterior new tissue in reconstitution of 1/6 pieces in water at room temperature. Fig. 6, 48 hours after section. Fig. 7, 3 days after section. Fig. 8, anterior piece, 5 days. Fig. 9, piece from posterior region of first zooid, 5 days. Fig. 10, anterior piece, 8 days. Fig. 11, piece from posterior region of first zooid, 8 days.

of the pieces the difference in growth rate is also distinct and in both the rate of growth of new tissue is evidently lower at the posterior than at the anterior ends. Figures 10 and 11 show the characteristic differences in pieces from anterior and posterior levels of the first zooid eight days after section. In the anterior piece (Fig. 10) the scale of organization of the head is larger and the amount of new tissue posterior to the level of the eyes is less and the amount of posterior new tissue is greater than in the posterior piece (Fig. 11). In the posterior zooid region the scale of organization of the head again becomes larger and the forms which develop resemble Figures 10 rather than Figure 11. At this stage, eight days after section the new tissue of the head has differentiated and ceased or almost ceased to grow, but growth of the posterior new tissue continues, though more slowly than earlier, until the posterior outgrowth may often become larger than the anterior.

In pieces below one eighth to one tenth of the total body length from the more posterior levels of the first zooid development of a head is sometimes inhibited by physiological conditions originating at the posterior cut end of the piece. These case and the factors concerned will be considered elsewhere. At present it need only be noted that in pieces in which the head is completely inhibited the growth rate of the posterior new tissue may be more rapid than in pieces which develop a head, apparently because in the absence of the head the posterior region develops as a new zooid and fission often takes place a week or two after section. But in pieces which give rise to complete normal individuals the higher

growth rate of the anterior end is characteristic of *P. gonocephala* and present but less marked in *P. dorotocephala*. This difference in growth rate indicates the presence of a physiological gradient which determines growth rate and which corresponds to the longitudinal susceptibility gradient in the body of the animal.

When reconstitution takes place in low, non-lethal concentrations of KCN (m/100000-m/400000) and of various other inhibiting agents certain modifications of the growth rate occur which are of considerable interest physiologically. The data of four series of such experiments are given.

Series 40. Ten animals 10–12 mm. in length from a single stock were cut into 1/6 pieces (A–F, Fig. 12). Each lot of ten pieces representing approximately a particular body level underwent reconstitution in a closed ERLENMEYER flask in KCN m/100000, pH 7.8, the flask being filled with the solution except for an air space of 10–15 mm. depth below the stopper. The solution was renewed every 3–4 days from freshly prepared KCN solution and the flasks were kept at room temperature.

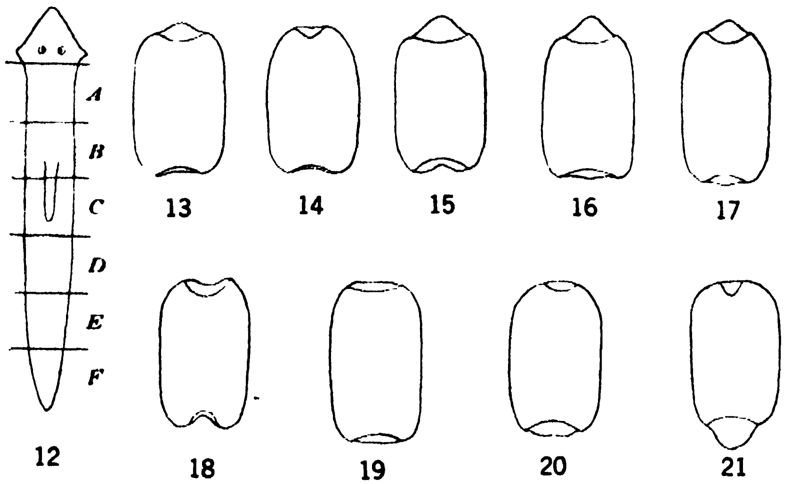
Series 57. Ten animals 16–18 mm. in length cut into 1/6 pieces and each lot of 10 pieces together in KCN m/100000, pH 7.8 as in Series 40 and under similar conditions.

Series 41. Five animals 10–12 mm. in length cut into 1/6 pieces. All pieces together without distinction of body level in KCN m/200000, pH 7.6 under same conditions as preceding series.

Series 42. Similar to Series 41 except that in KCN m/400000, pH 7.6. Kept under same conditions as preceding series.

The numerical data of these series are given below in Tables II and III but before turning to these the most important results must be described. In Series 40 there is no appreciable growth of new tissue during the first 10–12 days in KCN but after 16 days more or less new tissue is present at the anterior end while growth at the posterior end is almost completely inhibited. In more than half the pieces the difference in amount of anterior and posterior outgrowth is distinctly greater (Fig. 13) than in controls in water (Figs. 6, 7) and even in pieces in which anterior growth is inhibited to a considerable extent posterior growth is still more completely inhibited (Fig. 14).

After 31 days in KCN the difference between anterior and posterior new tissue has become even greater in two thirds of the pieces (Figs. 15–17), but in some pieces from the posterior region of the first zooid anterior growth is more inhibited than in the pieces from more anterior levels so that in these posterior pieces the difference between anterior and



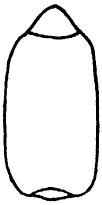
Figs. 12–21.—Reconstitution in KCN m/100000. Fig. 12, diagrammatic outline indicating approximate levels of 1/6 pieces, A–F. Figs. 13–21, growth of anterior and posterior new tissue in 1/6 pieces in KCN m/100000 (Series 40, 57). Figs. 13 and 14, 16 days after section. Figs. 15–21, 31 days after section.

posterior outgrowth is less or absent (Figs. 18, 19).

In Series 57 from somewhat larger animals also in KCN m/100000 pieces from the anterior region and the posterior zooid, more than half the total number of pieces, show after 31 days in KCN, like Series 40, a relative increase of anterior over posterior outgrowth (Figs. 15–18) as compared with controls in water. But in pieces from the posterior levels of the first zooid there is in most cases little anterior outgrowth and the posterior outgrowth is equal to, or larger than the anterior (Figs. 20, 21). Consideration of the probable significance of these cases is postponed until the other data have been presented.

In Series 41 in which the 1/6 pieces from different levels were all together in KCN m/200000 for 31 days two thirds of the pieces show greater anterior than posterior outgrowth and the difference is greater than in Series 40 and 57, that is, the anterior outgrowth is on the average larger than in the higher concentration of KCN while the posterior outgrowth is still to a large extent inhibited (Figs. 22, 23). Moreover, there are no cases in this series in which the posterior outgrowth becomes larger than the anterior while the pieces remain in KCN.

The pieces of Series 42 in KCN m/400000 without separation of different body levels develop much more rapidly than those in the higher



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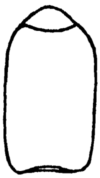

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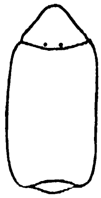

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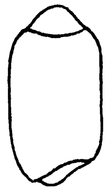

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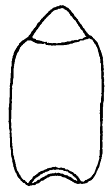

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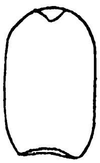

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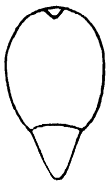

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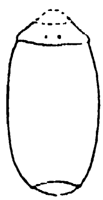

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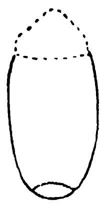

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Figs. 22–28. — Growth of new tissue in 1/6 pieces in KCN m/200000 and m/400000. Figs. 22 and 23, m/200000 16 days after section (Series 41). Figs. 24–28, m/400000 13–27 days after section.

Figs. 29, 30. — Reversal of difference in susceptibility in killing concentrations of KCN after reconstitution in low concentration of KCN. The head, which is least susceptible in low concentrations is most susceptible in killing concentrations.

concentrations. After 13 days in KCN almost 90 per cent show much larger anterior than posterior outgrowth and some have already developed eyes (Figs. 24, 26). About ten per cent show a condition like Figure 27 and in a single piece the posterior outgrowth is larger than the anterior (Fig. 28). After 17 days in KCN the pieces were returned to water and after 24 hours in water those which had developed heads were tested as regards their susceptibility to killing concentrations of KCN (m/10000 and higher). In every one of ten pieces in KCN m/10000 the head was the most susceptible region of the individual and disintegrated while the rest of the piece was intact and living (Figs. 29, 30). In some pieces the posterior new tissue disintegrated earlier, in some later than the old tissue. In other killing concentrations the head was always the most susceptible region. Pieces of the other series which were removed to water after 31 days in KCN and which developed heads during the next eight days gave the same result in killing concentrations.

It appears then from these experiments, first, that the anterior new

tissue is in general less susceptible to the low concentrations of KCN (m/100000–m/400000) than the posterior new tissue; second, that this difference in susceptibility between anterior and posterior new tissue increases with decrease in concentration within the range of concentrations used; third, to higher gradually lethal concentrations the anterior new tissue is always the most susceptible region of the animal. In other

TABLE II.

Reconstitution of 1/6 pieces in KCN and of controls in water. Different levels of body separated.

Series	Number of animals	Length in mm.	Medium	Days after section	Condition	Body levels A	B	C	D	E	F
40	10	10-12	KCN m/100000	16	living	8	8	10	10	10	9
					anterior larger	8	2	2	4	10	—
			KCN m/100000	31	living	6	6	6	6	9	6
					anterior larger	4	3	2	4	9	—
			water 8 days	39	living	4	6	6	6	5	6
					normal	4	—	1	2	5	6
					inhibited	—	1	1	1	—	—
					acephalic	5	4	2	—		—
57	10	16-18	KCN m/100000	31	living	10	8	10	10	9	9
					anterior larger	10	8	2	2	5	—
			water 8 days	39	living	10	8	10	10	9	9
					normal	1	-	—	4	5	3
					inhibited	9	—	-	3	4	5
					acephalic	—	8	10	3		—
Control	10	16-18	water	3	living	10	10	10	10	10	10
					anterior larger	10	10	10	10	10	—
				12	living	10	10	10	10	10	10
					normal	10	9	9	10	10	10
					inhibited	—	1	1	—	—	—

words, the developing head, which is less susceptible to low concentrations which retard or inhibit growth is more susceptible to higher concentrations which gradually kill than the posterior new tissue or the old tissue.

Table II gives the numerical data of Series 40, 57 and a control in water. The first five columns of the table need no explanation. The sixth column headed, "Condition" gives the different conditions of the pieces distinguished for present purposes. The line headed, "anterior larger" gives the numbers of pieces in which the anterior new tissue shows a distinctly larger outgrowth than the posterior at the time of record. The terms, "normal," "inhibited," "acephalic," in the sixth column distinguish the types of head finally reconstituted. The normal heads are heads of normal form with two symmetrically localized eyes of equal or approximately equal size and two cephalic lobes lateral to the eyes. The inhibited heads include all other forms of heads. These show various degrees of inhibited development of the median head region and are very similar to the inhibited head forms in *P. dorocephala* described as teratophthalmic, teratomorphic and anophthalmic (see, for example, CHILD, 1921). The acephalic forms are those in which there is no growth of new tissue at the anterior end beyond the filling in of the greatly contracted cut surface. The columns A–F under the column heading "Body levels" give the numbers of pieces at each level showing the conditions described in the sixth column.

In both experimental series of Table II the number of pieces in which the anterior outgrowth becomes larger than the posterior in KCN decreases from the anterior to the posterior region of the first zooid (pieces A, B, C) and increases again in the posterior zooid region (D and E pieces). The F pieces have no posterior cut surface. As regards the final condition at 39 days, after 8 days in water, both series show similar regional differences. Head development is least inhibited in the anterior region (A pieces) and the posterior zooid region (D, E, F). Evidently the degree of head development at different levels varies in the same way as the development of larger anterior outgrowth in KCN. The control series of Table II gives the usual result for 1/6 pieces in water. The anterior outgrowth is larger in all and all except one each in the B and C pieces give rise to normal heads. Little variation from this result occurs. In some series all heads are normal, in some two or three B and C pieces may show a slight degree of teratophthalmia: deaths occur rarely. But even though all heads may be normal in such series the rate of head development and size of head decrease from A to B and C pieces (Figs. 8–11) and usually show some increase in D and more in E and F. These

differences are characteristic during the first five to eight days of reconstitution but later, as the heads which develop more rapidly complete their growth those which develop more slowly may gradually attain the same size. Evidently the differences in rate of head development and size of head during the earlier stages of reconstitution parallel the non-specific differences in susceptibility to killing concentrations of agents. In view of all the facts it is evident that the regions of the body which are least inhibited in the very low concentrations of KCN are in general the regions of most rapid growth and development in reconstitution which are most susceptible to killing concentrations.

As regards the character of the anterior end which develops at different levels of the body, both experimental series of Table II show that head development is most inhibited in the B and C pieces, that is, at the more posterior levels of the first zooid which are the least susceptible regions in killing concentrations of KCN. In Series 40 most, and in Series 57 all of the B and C pieces are acephalic. These pieces show in general a higher growth rate and larger size of the posterior outgrowth than pieces which develop heads. When head development is completely inhibited in water pieces also show this higher growth rate and larger size of posterior outgrowth. In *P. dorotocephala* the posterior half, more or less, of the acephalic form represents a new zooid which arises in consequence of the absence of a head and grows more rapidly than a posterior end which is not a zooid. These acephalic forms often undergo fission even though only 2–3 mm. in length (CHILD, 1911, p. 227). When a head is present fission never occurs in animals as short as this. Evidently the same conditions exist in the acephalic forms of *P. gonocephala* for short acephalic forms of this species also undergo fission. The point of chief interest at present, however, is that in such pieces the posterior outgrowth which has a higher growth rate than the anterior in water is less inhibited than the anterior outgrowth in the low concentrations of KCN.

And finally it may be noted that in Series 40 of Table II a total of 67 per cent of the living pieces develops heads and 54 per cent are normal, that is, fully developed heads, while in Series 57 61 per cent develop heads but only 23 per cent are normal. The animals of Series 57 are larger and physiologically older and undoubtedly have a lower respiratory rate than those of Series 40 (HYMAN, 1919 c, ROBBINS and CHILD, 1920) and are less susceptible in killing concentrations. In short, the same reversal of susceptibility differences appears between individuals of different physiological age as between different regions of the body.

The individuals which are most susceptible to killing concentrations are least inhibited in reconstitution in the very low concentrations and vice versa.

TABLE III.

Reconstitution of 1/6 pieces in KCN. Different levels of body not separated. Posterior piece (F, Fig. 12) discarded. Five pieces, A–E from each of five animals 10–12 mm. in length in each series.

Series	Number of pieces	Medium	Days after section	Condition	Numbers	Per cents of living
41	25	KCN m/200000	31	living	22	
				anterior larger	15	68
		water 8 days	39	living	19	
				normal	10	52.6
				inhibited	4	21
				acephalic	5	26.3
42	25	KCN m/400000	13	living	25	
				anterior larger	22	88
		KCN m/400000	16 27	living	21	
				normal	13	62
				inhibited	7	33
				acephalic	1	5

Table III differs slightly in form from Table II because the pieces from different body levels are not separated, also the numbers of pieces showing the different conditions are given in the last column as per cents of the number of living pieces at the time of record. In Series 41 in KCN m/200000 the anterior outgrowth is larger than the posterior in 15 of 22 living pieces, 68 per cent and in Series 42 in KCN m/400000 in 22 of 25 living, 88 per cent. Moreover, as was shown above (p. 333), in both these series the anterior outgrowth is not only larger than the posterior but relatively larger than in water and the difference in relative size is greater in the lower concentration of KCN (Figs. 22, 23, Series 41; Figs. 24-26, Series 42; Figs. 8, 9, water). It was also shown that this anterior outgrowth is more susceptible and dies earlier in the high, killing con-

centrations of KCN. As regards character of the anterior end Table III gives only totals of normals, inhibited and acephalic but in the lower concentration these show, as might be expected a larger total number of heads (13+7, 95 per cent of living) and a larger number of normal heads (13, 62 per cent of living) than in the higher concentration (total heads, 14, 73 per cent of living; normals 10, 52.6 per cent of living. In Series 42 the whole development of the heads took place in KCN in 16–27 days but in Series 41 and in Series 40 and 57 of Table II the pieces were removed to water after 31 days.

DISCUSSION

Fission. The occurrence of fission at a definite level or at two definite levels in many large animals, the differential susceptibility, both in killing concentrations and in low concentration of KCN, as shown in the development of anterior new tissue in pieces and in the differentiation of the head all argee in indicating that the posterior region of *P. gonocephala*, like that of *P. dorotocephala* (CHILD, 1910, 1911, 1913 a) is physiologically marked off from more anterior regions as one or more developing zooids, although the morphological delimitation is not evident. With slight stimulation independent contraction of a posterior zooid may often be observed. The posterior zooid apparently originates in consequence of a certain degree of physiological isolation in the posterior region of the body which in turn is correlated with the rate of growth in length. The chief factor in the physiological isolation is probably the nervous system. As growth in length of the body takes place the longitudinal nerve cords must undergo differentiation of new parts and such development extends the range of the dominance of the head. Apparently, however, the differentitation and functional development of the nervous system may not keep pace with the growth in length of the body and some degree of physiological isolation may occur in the posterior region since that is the region in which growth in length is most rapid. In a region so isolated physiologically some degree of reorganization of the nervous system must occur resembling that which takes place in a physically isolated piece, though in *Planaria* it does not proceed very far until separation takes place. The rate of development of the posterior zooid and the length at which fission occurs depend on the degree of isolation. When growth in length is rapid the length attained before fission is in general less than when growth is slow. After the animals attain a certain size growth in length is almost entirely confined to the posterior zooid region and as this elongates a second posterior zooid

may arise through the physiological isolation of another region posterior to the first isolation. The head region of the first posterior zooid is very little developed and its range of dominance is short, consequently the second posterior zooid and the second fission plane develop at only a short distance posterior to the first. After this second posterior zooid is delimited fission may occur at either of the two fission planes, though it more commonly takes place at the anterior of the two.

The region of the fission plane is probably a region of physical weakness because of the varying conditions to which it is subjected. As one moment, for example, it may be more or less subordinate to the head of the animal, at another it may be influenced by the zooid posterior to it. It must, therefore, approach to some exent the condition of an indifferent zone between the two zooids.

The writer has not observed the act of fission in the Japanese planarian but it has often been seen in *P. dorotocephala*. There it is initiated by a motor reaction of the posterior zooid independent of anterior regions. The posterior zooid attaches to the substratum while the anterior part of the body attempts to continue forward movement, with the result that the region just anterior to the attached zooid becomes elongated and finally ruptures. In acephalic forms fission may occur without forward locomotion by the contraction longitudinally in opposite directions of the posterior zooid and the anterior headless part. Undoubtedly the act of fission in the Japanese species is essentially similar to that described.

The increase in the frequency of fission following removal of the head of the animal evidently results from a decrease in dominance over the posterior zooid region. In consequence of this decreased control a greater degree of physiological isolation exists in the posterior zooid region and as locomotor activity increases with the development of the new head fission is likely to occur, but if it does not occur before the head attains a certain stage it usually does not occur unless the head is again removed or its activity decreased, or unless the animal is fed and increases in length. There is every reason to believe that the changes which induce fission following removal of the head are primarily changes in the nervous system. When the cephalic ganglia and the chief sense organs are removed the nervous stimuli which reach the posterior zooid region from the anterior end undoubtedly become less frequent or less intense or both. Under these conditions reorganization in the posterior zooid must progress more rapidly and it becomes more independent and the probability of fission is increased. How much reorganization of the postcephalic nerve cords is

brought about by development of new cephalic ganglia after removal of the original ganglia is not known, but within seven or eight days after removal of the head the dominance of the new head appears to be as complete as that of the original head.

Differential Susceptibility. The data show that the regions of the body which are most susceptible and most rapidly killed in the high concentrations of KCN are in general least inhibited in growth and development in low concentrations and vice versa. This holds not only for widely separated regions of the body but for anterior and posterior ends of single isolated pieces no more than one sixth of the total body length. It also holds for still shorter pieces but such pieces are not considered in the present paper.

In so far as differences in physiological condition are quantitative and non-specific for different agents the individual or body region with a high metabolic rate and with protoplasmic conditions corresponding to that rate is more susceptible to concentrations above the limit of tolerance because the more rapidly the changes characteristic of life are proceeding the sooner is the point reached at which they are altered or inhibited by the action of the agent. On the other hand, if the agent can be oxidized, reduced or otherwise altered by the living protoplasm so that it becomes non-toxic or less toxic, it is to be expected that the individual or region of the body with the higher rate of metabolism will dispose of the agent more rapidly than the less active individual or region. This more rapid disposal of the agent, probably by oxidation in the case of KCN, or the more rapid compensation of the changes brought about by it, must be the physiological basis of the lesser degree of inhibition of the anterior outgrowth of new tissue in the isolated pieces of *P. gonocephala* in the low concentrations of KCN. That this lower susceptibility is not specifically characteristic of anterior new tissue is indicated by those pieces of Series 57 from the more posterior levels of the first zooid (Table II, Series 57, B and C) in which the posterior outgrowth has a higher growth rate because of inhibition of head development and is less inhibited by KCN than in other pieces (p. 336).

If acclimation or acquirement of increased tolerance to an agent in low concentration or dosage occurs, it is to be expected that the physiologically more active individual or region will acclimate more rapidly or to a greater extent than the less active. Whether the anterior outgrowths of the isolated pieces actually underwent acclimation in the KCN experiments cannot be determind with certainty, but the exaggeration of the

difference between anterior and posterior growth rate as compared with pieces in water, particularly in Series 42 in KCN m/400000 and the differentiation of heads with eyes in the KCN with almost complete inhibition of posterior growth (Figs. 24–26) make it probable that some degree of acclimation has occurred in the anterior new tissue. Similarly, the fact that in many pieces of Series 40 and 57 there is almost no growth of new tissue during the first two weeks in KCN, but a considerable growth at the anterior end during the second two weeks indicates that some degree of acclimation may have occurred. But whether or not acclimation occurs in any of the experiments the experimental data demonstrating a reversal of differential susceptibility in different ranges of concentration of KCN are important in two ways. First, as regards the physiological basis of the differences in susceptibility and their reversal, the only possible conclusion seems to be that they depend on quantitative, rather than qualitative or specific differences in physiological condition. Second, they demonstrate the necessity of determining the effects of widely different ranges of concentration or dosage in investigating the action of external agents on living protoplasm.

Reversal of differential susceptibility has been observed in *P. dorotocephala* in KCN, alcohol, ether acid and certain ranges of low temperature (CHILD, 1912, 1913, 1914 a and unpublished data). In both species such reversal may appear between large and small individuals, between different body-levels of the same individual or between anterior and posterior ends of isolated pieces.

SUMMARY

1. The Japanese planarian known as *Planaria gonocephala* resembles *P. dorotocephala* and *P. maculata* in the physiological demarcation of the posterior region of the body of larger animals as one or more zooids which result from a partial physiological isolation of this region.

2. In the larger animals in which more than one posterior zooid may be present fission usually separates the whole posterior zooid region from the anterior zooid but occasionally fission occurs between the second and third zooid.

3. Fission occurs more frequently in individuals of a given length when growth has been rapid than when it has been slow. As a possible basis for this difference it is suggested that when growth in length is rapid, differentiation of the nervous system and the extension of dominance associated with it may not keep pace with the increase in length.

4. Removal of the head increases the frequency of fission in large animals and often induces fission in small animals of lengths which very rarely or never undergo fission when intact and in normal environment.

5. The presence of two zooids in the posterior fission pieces of large animals is indicated by the fact that they often undergo a second fission within a few days after the first fission which separated them from anterior body-regions. If such fission does not occur before the new head attains a certain stage it may often be induced by removal of this head.

6. Differential susceptibility to concentrations of the various agents used which kill gradually is in general similar to that in *P. dorotocephala.* In the anterior zooid the susceptibility of the body surface and body wall and probably the parenchyma and nervous system decreases from the anterior end posteriorly to the level of fission.

7. The posterior zooid region is apparently somewhat more susceptible to the stimulating action of alkaline and the depressing action of acid agents and may also show a slightly greater tolerance for the lower killing concentrations. Because of these characteristics its relative susceptibility varies somewhat and may be either higher or lower than that of the posterior parts of the anterior zooid.

8. The extreme posterior tip of the body is a region of high susceptibility to killing concentrations, probably because it is a region of growth in length, but functional activity may contribute to this high susceptibility.

9. The differences in susceptibility between lateral and median and between dorsal and ventral surfaces show some degree of specificity for particular agents which is evidently a result of their differentiation in different directions.

10. In slightly acid distilled water the susceptibility gradients are similar to those in other slightly acid agents. Survival time in distilled water varies directly with number of individuals and inversely with volume of water. Death of some individuals in distilled water usually prolongs the lives of others if the water is not changed. Some individuals live for at least six weeks in distilled water with frequent change of water.

11. In isolated pieces in water the growth rate of new tissue is in general higher at the anterior than at the posterior end of the piece.

12. In isolated pieces kept continuously in very low concentrations which do not entirely inhibit growth of new tissue at the cut ends (m/100000–m/400000) growth at the anterior end is less inhibited than at the posterior end. In the lowest concentration used (m/400000) normal

heads may develop in the solution while growth at the posterior ends of the same pieces is almost completely inhibited.

13. The fact that in KCN m/100000 during the first two weeks all growth of new tissue is almost completely inhibited while during the next two weeks considerable growth occurs at the anterior end but posterior growth is still to a large extent inhibited suggests that some degree of acclimation takes place at the anterior end. The fact that the difference in growth rate and size between anterior and posterior new tissue is greatly increased in the low concentrations of KCN also indicates the occurrence of some degree of acclimation at the anterior end.

When the pieces which have developed in low concentrations of KCN are placed in killing concentrations the anterior ends, which were the least susceptible regions in the low concentrations are the most susceptible in the high concentrations.

15. The differential susceptibility to gradually killing concentrations of agents which is characteristic of the anteroposterior axis of intact animals, the difference in growth rate of anterior and posterior new tissue outgrowths in isolated pieces in water and during reconstitution in low concentrations of KCN and the reversal of the difference in susceptibility of these outgrowths in low and high concentrations all agree in indication the existence of a quantitative gradient in physiological condition in the longitudinal axis.

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THE AGE AND GROWTH OF THE LIMPET (*ACMAEA DORSUOSA* GOULD).[1]

BY

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(With 13 text-figs.)

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The present paper deals with the growth of *Acmaea dorsuosa*, GOULD, chiefly from an ecological standpoint, which has been observed since 1930 at the Asamushi Marine Biological Station.

During the course of my studies I have been kindly guided by Prof. Dr. S. HATAI, to whom I here wish to express my sincere thanks.

1. MATERIAL.

I have attempted to collect all the colony forming individuals from each given spot. *Acmaea dorsuosa* colonizes in the spring and summer and breaks up during the autumn and winter seasons as I have already reported ('31). Since they do not migrate more than five to six metres after breaking up of the colony, it will be safe to assume that all the individuals that participated in forming the original colony are exposed to the same environmental conditions every year.

On the growth of *Patella vulgata*, ORTON ('28) stated that the shell-shape or $(L+B)/2H$ varies according to their habitats, and consequently, I also have noticed similar changes with *Acmaea*.

NOMURA ('26) noticed the influence of coastal waves on the growth of *Littorina sitchana*, PHIL. but I have noticed no such influence with *Acmaea*, since, unlike the former the latter inhabits places where the coastal waves are non-effective.

Favorite localities where the limpets are abundantly found are the islands Ohshima, Mourajima and Yunoshima which are about 12.5 Kilo-metres, 3.2 Kilo-metres to the north and one Kilo-metre to the south-west from the Station.

Fortunately, *Acmaea* is not used as food and thus they live undisturbed and grow under very natural circumstances.

[1] Contributions from the Marine Biological Station, Asamushi, Aomori-Ken. No. 87.

II. METHOD.

For the study of growth the following three methods were used.

1. *Direct observation.* Several stationary colonies in natural habitats were chosen and the individuals there were measured occasionally. This method was applied by ORTON for *Cardium edule* ('26), *Ostrea edulis* ('28) and *Patella vulgata* ('28), while GUTSELL ('30) used the same method for *Pecten irradians.*

2. *Annual ring method.* This method was chiefly used in determining the age of *Acmaea dorsuosa*, which shell shows the annual rings clearly. This annual ring method has been employed by various investigators for various other molluscs, for instances; CROZIER ('14, '18) for *Chiton tuberculatus*, WEYMOUTH ('23) for *Tivela stullorum*, WEYMOUTH, MCMILLIN and HOLMES ('25) for *Siliqua patula* (DIXON), WEYMOUTH and MCMILLIN ('30) again for *Siliqua patula* (DIXON), WEYMOUTH and THOMPSON ('30) for *Cardium corbis*, MASTYN.

Ages of fresh-water mussels were also determined by this method.

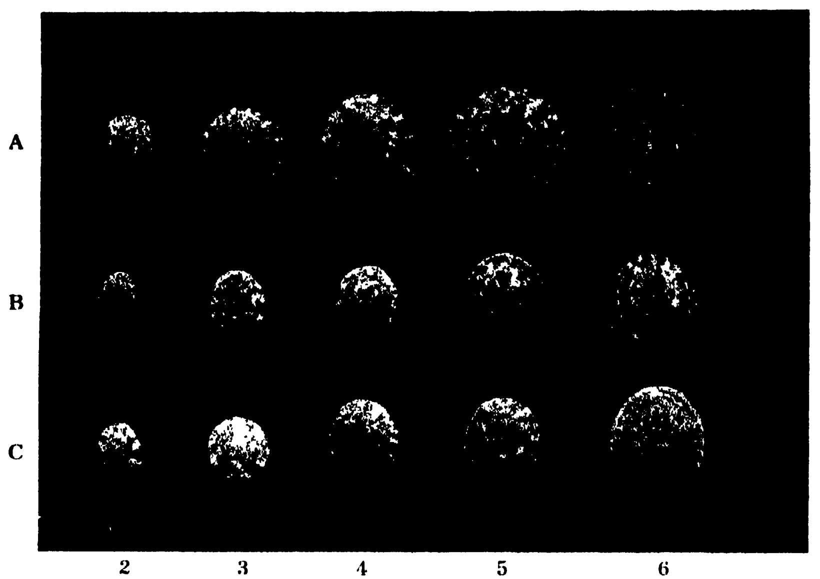

Fig. 1. Annual rings of *Acmaea dorsuosa*.
No. 2, 3, 4, 5 and 6 represent the ring numbers.
A. ....the limpets found on the wet place of Mourajima.
B. ....the limpets found on the drier place of Mourajima.
C. ... the limpets found on the drier place of Ohshima.

Among those, are the observations by CHAMBERLAIN ('30) for the growth of *Lampsilis anodontoides* (LEA) and *Lampsilis siliquoidea*.

3. *Shell measurements.* Since the animals under normal circumstances grow in proportion with age, the age of the shellfish can be indirectly estimated from the shell-measurements. This method was used by NOMURA ('26, '28) for *Littorina littorea*, L., *Littorina sitchana*, PHIL, *Sphaerium heterodonta*, PILS, *Limnea japonica*, JAY, *Viviparus japonica var. iwakawai*, PILS, *Tapes philippinarum*, ADS. and RVE, *Cythera* (*Meretrix*) *meretrix*, L. MAKIYAMA ('30) measured the shell-dimensions of *Glycymeris yessoensis* OHUE ('31) of *Thiala liberata*, GOULD, TAKANO ('32) of *Nassarius hispidus*, ADAMS, *Umbonium giganteum*, LESSEN, *Pythia striata*, REEVE, *Monodonta labio*, L, and *Nerita plicata*, L.

Acmaea dorsuosa exhibits the annual rings as are shown in Fig. 1. Whether or not the so-called "annual ring" represents the age as I assumed, I followed the growth of the limpet at Hadakajima, one of the islands near the Station, and also with some which were kept in the laboratory since last spring. It was actually noted that one ring is added in one year. But among the older limpets, age which was older than 7 or 8 years in which the ring numbers are not very distinct were omitted from the data.

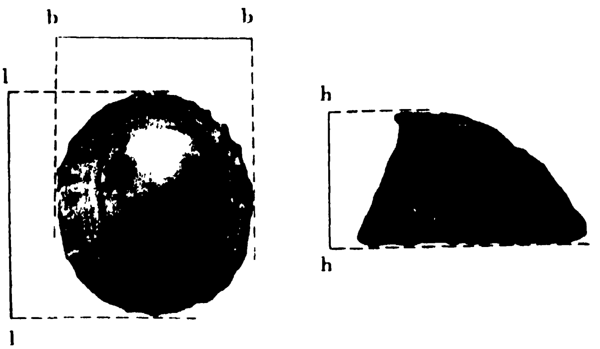

Fig. 2. Method of measurement of *Acmaea dorsuosa*.
l -l - length, b- b -breadth, h--h = height.

The length, breadth and height of the shell were determined in the manner as are shown in Fig. 2 by means of caliper which scales enable one to read accurately to the 20th of a milimetre.

III. GROWTH.

1. *Shell-length* (L) *in relation to age.* *Acmaea dorsuosa* having the

same number of rings, show considerable variation in respect to their shell-lengths. The ranges of variability are shown in Fig. 3.

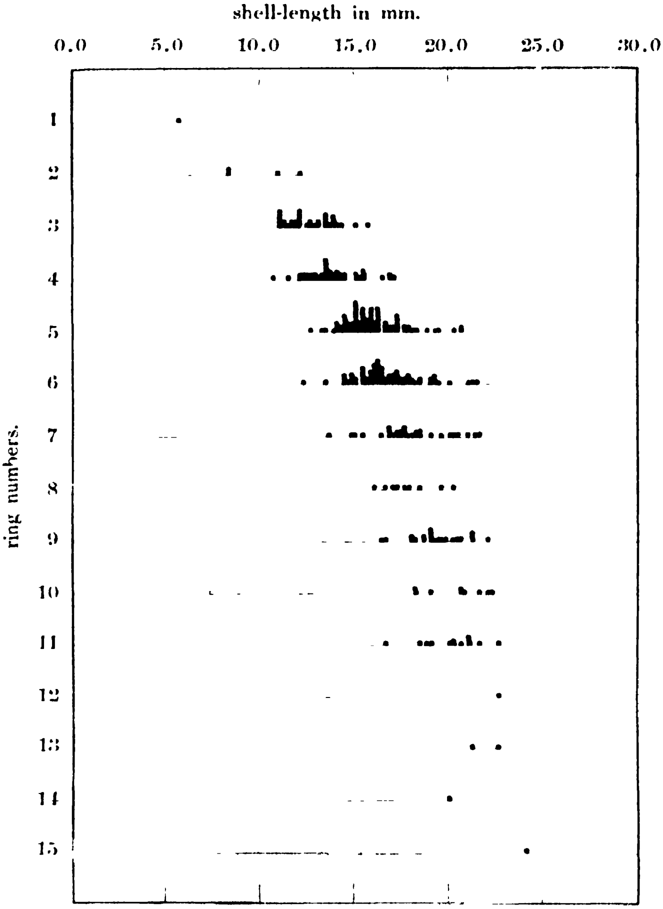

Fig. 3. Variability of shell-length in *Acmaea* having the same number of rings (Mourajima)

If, however, the mean value of the shell-length in each age is taken, we obtain some continuous growth curve as is shown in Fig. 1, which is based on the data given in Table I.

As shown in Fig. 1 the general form of the growth curve is similar as are most of the growth curves given by other animals; that is, the growth rate in the younger exceeds that of the older shells.

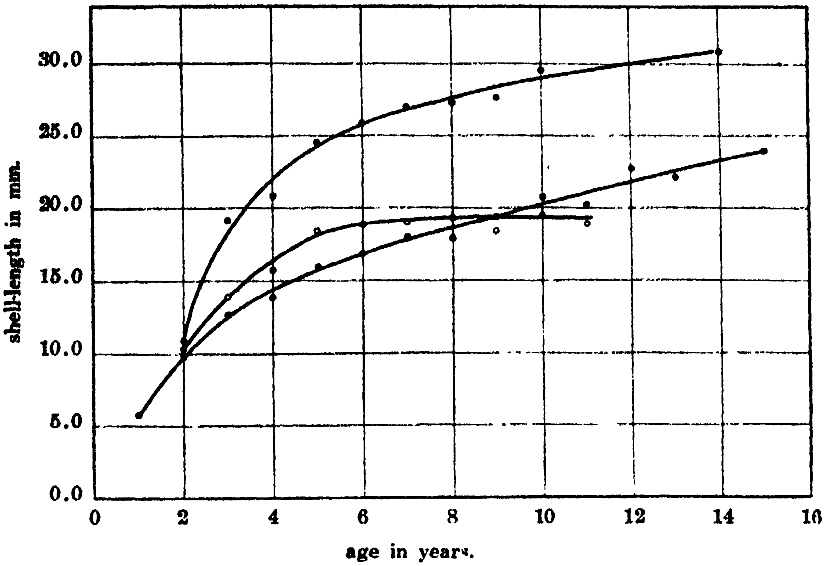

Fig. 4. Shell-length in relation to age. (Ring number)
o——o Ohshima
●——● Mourajima (wet place)
•——• Mourajima (drier place)

TABLE I. Shell-length in relation to age.

No. of rings. or age in year	Ohshima (drier place)		Mourajima (drier place)		Mourajima (wet place)	
	No of individuals	Shell-length in mm.	No. of individuals	Shell-length in mm.	No. of individuals	Shell-length in mm.
1	0		1	5.8	0	—
2	6	10.4	4	9.9	3	10.9
3	109	14.0	35	12.6	22	19.2
4	103	15.8	42	13.8	43	20.8
5	44	18.4	79	16.0	35	24.5
6	37	18.9	71	16.8	33	25.9
7	13	19.0	29	18.0	12	27.0
8	8	19.3	9	18.0	1	27.4
9	13	18.4	23	19.5	2	27.7
10	26	19.5	9	20.7	2	29.7
11	21	18.8	13	20.1	1	25.3
12	1	18.6	1	22.7	0	—
13	2	18.3	2	22.1	0	—
14	0	—	1	20.1	1	30.9
15	0	—	1	24.0	0	—

When the logarithmic values are plotted, we obtain the curves as are given in Fig. 5 in which the two phases of the growth rate are clearly

shown, one phase represented by the limpets younger than and the other phase by the older than about 6 or 7 years of age.

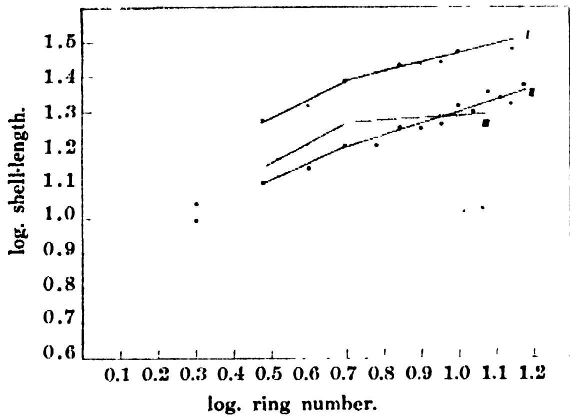

Fig. 5. Logarithmic shell length.
I. Mourajima (wet place)
II. Mourajima (drier place)
III. Ohshima.

Since the difference between the ordinates on the two consecutive points in Fig. 4 corresponds to the differential of the growth curve, it will repre-

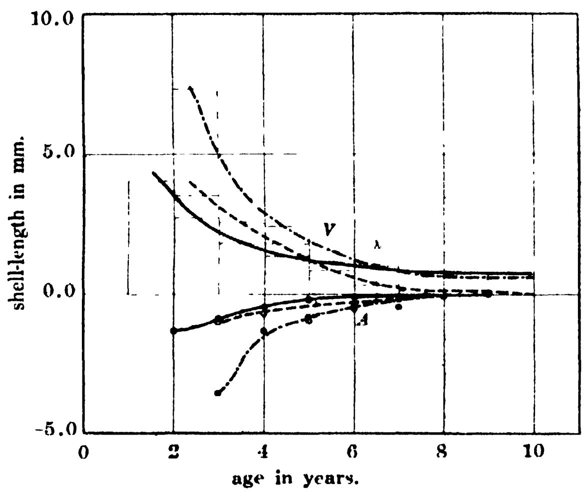

Fig. 6. The velocity (V) and the acceleration or absolute growth (A) of *Acmaea dorsuosa.*
-- ---- Ohshima
——— Mourajima (drier place)
—·—·— Mourajima (wet place)

sent the growth rate with respect to the age (Fig. 6 V). Similarly the second differential of the growth rate will represent the acceleration of the growth as is shown in Fig. 6 A.

From the above it becomes clear that, the limpet which inhabits a wet place has a faster growth rate than the one which inhabits a drier place. For example, the ratio of shell-length of the former to the latter is in average 1.52 (1.50–1.54) for individuals of 3 years old to 8 years old in favor of the limpets inhabiting a wet place (see Table I).

2. *Shell-length and shell-breadth in relation to age.* In the following the relation between the breadth and length, B/L, to age is given, *where L = shell-length and B = shell-breadth. The ratio of B/L is shown in Table II and in Fig. 7.

From the above the values of B/L in the limpet are practically constant with the individuals older than 3 years, or even when all the age groups

TABLE II. B/L in relation to age.

Ring No. or age in years	Mourajima				Ohshima			
	No. 1		No. 2		No. 1		No. 2	
	No.	B/L	No.	B/L	No.	B/L	No.	B/L
1	1	0.78	0		0		0	
2	4	0.79	3	0.82	4	0.77	2	0.79
3	35	0.80	19	0.84	85	0.79	24	0.80
4	43	0.80	32	0.83	78	0.79	25	0.80
5	79	0.80	29	0.84	33	0.79	11	0.80
6	71	0.80	25	0.84	26	0.79	11	0.79
7	29	0.80	12	0.84	11	0.79	2	0.81
8	9	0.80	1	0.87	5	0.78	3	0.80
9	23	0.81	2	0.85	12	0.78	1	0.81
10	9	0.80	1	0.84	24	0.79	2	0.79
11	13	0.82	1	0.83	21	0.79	0	
12	1	0.84	0	--	3	0.78	0	
13	2	0.80	0	-	2	0.79	0	
14	0	---	1	0.81	0		0	
15	1	0.79	0	--	0		0	

Mourajima, No. 1....drier place, the rock faces north-ward.
No. 2....wet place, the rock faces north-ward.
Ohshima, No. 1... drier place, the rock faces west-ward.
No. 2... drier place, the rock faces south-west and differs about 20 metres from No. 1.

* Prof. E. NOMURA told me in private communication that the growth of *Acmaea dorsuosa* may be represented by the formula, $a = kb^x$, where "a" represents shell-length and "b" represents shell-breadth and "k" is the local constant and the shells collected from Yunoshima gave $b = 0.772\,a^{1.02}$.

Fig. 7. B/L in relation to age
○——○ Mourajima (wet place) ●——● Ohshima No. 1.
●- - -● Mourajima (drier place) ○- - -○ Ohshima No. 2.

are compared, it shows a fair degree of constancy. It is, however, to be noted that within the specimens collected from the same locality, the younger limpets, less than two years, show lower values of B/L than that of the older one, or in other words, the shape of the younger *Acmaea* has a rounder shell margin than in the older one.

TABLE III. Shell dimension, (L+B)/2 H.

Ring No. or age in years	Ohshima				Mourajima			
	No. 1		No. 2		No. 3 (drier place)		No. 4 (wet place)	
	No.	$\frac{L+B}{2H}$	No.	$\frac{L+B}{2H}$	No.	$\frac{L+B}{2H}$	No.	$\frac{L+B}{2H}$
1	0	—	0	—	1	2.65	0	-
2	4	2.49	2	2.30	4	2.58	3	2.69
3	85	2.34	24	2.24	34	2.50	19	2.52
4	77	2.26	25	2.08	42	2.49	30	2.44
5	33	2.21	11	1.94	79	2.30	28	2.29
6	27	2.00	11	1.85	71	2.21	25	2.24
7	11	1.88	2	1.83	28	2.10	12	2.22
8	5	1.83	3	1.72	9	1.92	1	2.11
9	13	1.71	1	1.69	23	1.88	2	1.96
10	23	1.68	2	1.55	9	1.80	1	2.05
11	21	1.63	0		13	1.81	1	1.54
12	1	1.47	0	—	1	1.68	0	—
13	1	1.58	0	—	2	1.67	0	—
14	0	—	0	—	1	1.30	1	1.65
15	0	-	0	—	1	1.57	0	-

And also the limpets which live on the drier place show lower values of B/L than those living in the wet place.

3. ***Variations in shell-shape in relation to age.*** Dimension of shell-shape, $(L+B)/2H$, is given by ORTON on *Patella vulgata*, where, L= shell-length, B=shell-breadth and H=shell-height. Since it is important and interesting from the ecological standpoint to study the variations in shell-shape, I have measured the values of $(L+B)/2H$ on *Acmaea dorsuosa*. The results are given in Table III and Fig. 8.

From Fig. 8 it will be seen that the value of $(L+B)/2H$ is higher in the younger limpet than in the older one. And this progressive reduction of the values with age is chiefly due to the greater growth in the shell-height. (It was already stated that B/L is practically constant and if $B/L=k$, then $(L+B)/2H=L(1+k)/2H$, and therefore the shell-height is chiefly responsible to the variations of shell-shape due to age.)

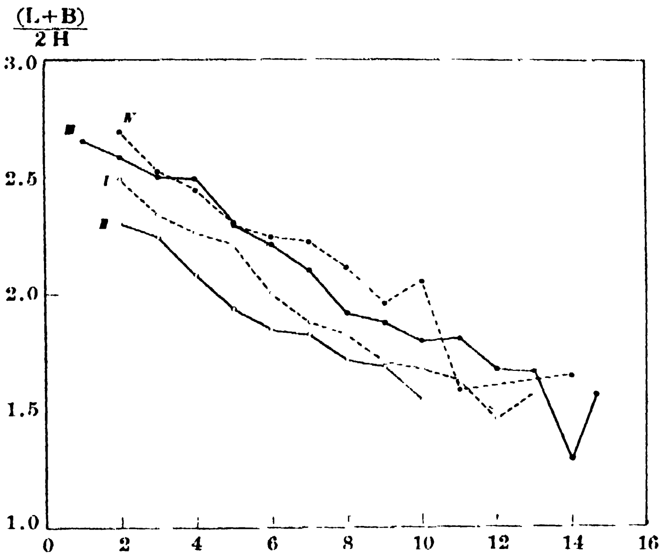

Fig. 8. $(L+B)/2H$ in relation to age.
I....Ohshima No. 1 III....Mourajima (drier place)
II....Ohshima No. 2 IV....Mourajima (wet place)

As shown in Fig. 8, the values of $(L+B)/2H$ obtained from the individuals collected from Mourajima are higher than those collected from Ohshima, and it follows then that the limpets living on Ohshima possess shell which height is comparatively higher than those on Mourajima.

However, when the limpets collected at the wet place are compared with those collected at a drier place the former exceeds that of the later, though the shell-heights are practically the same in both specimens. Consequently it follows that the limpet on the wet place is relatively lower in its shell-height than the limpet on the drier place with respect to given shell-length as ORTON already noted in *Patella vulgata*.

When the values log $(L+B)/2H$ are plotted on the corresponding logarithmic values of the ring number, we obtain the relation shown in Fig. 9, which shows that in the limpets younger than 4 years of age, the growth rate of shell-height is higher with the individuals on Ohshima than that of Mourajima, and after the age of 4 years, the growth rate of shell-height becomes about the same in the both specimens.

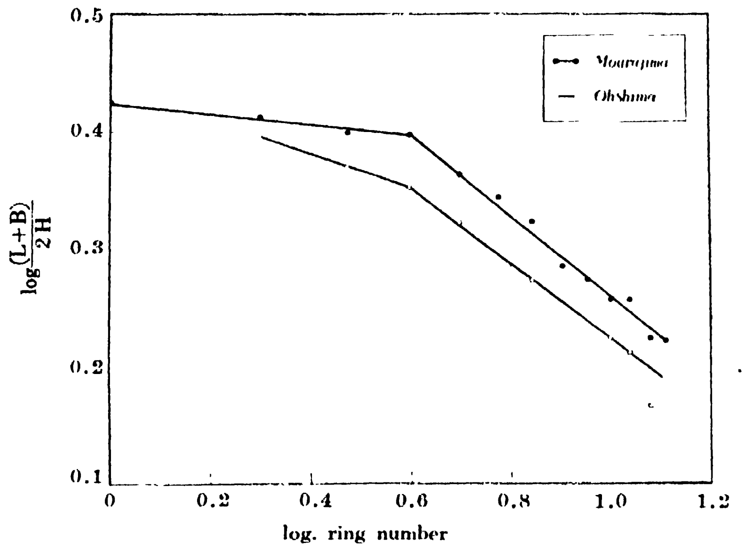

Fig. 9. Logarithmic shell-dimension, log $(L+B)/2H$.

4. *Growth of shell-weight with respect to the age and to the shell-length.* The growth of shell-weight with respect to the age is shown in Table IV and Fig. 10. We notice that the limpets found on the wet place are much heavier in the shell-weight than those found on the drier place.

The relation of shell-weight to the shell-length are shown in Table V and Fig. 11. In Fig. 11, we note that the shell-weight increases in association with the increase in the shell-length, but in the limpets smaller than about 16 or 17 mm. in the shell-length the increase in shell-weight is

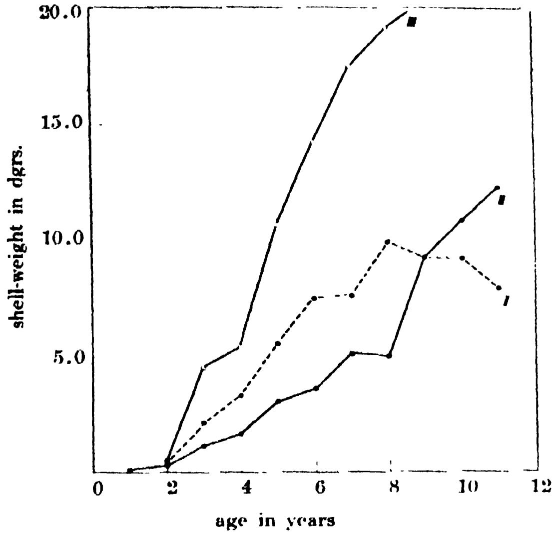

Fig. 10. Shell-weight in relation to age.
I ...Ohshima
II .. Mourajima (drier place) III . Mourajima (wet place)

TABLE IV. Shell-weight in relation to age.

Ring No. or age in years	Ohshima		Mourajima (drier place)		Mourajima (wet place)	
	No.	Shell-weight in dgrs.	No.	Shell-weight in dgrs.	No.	Shell-weight in dgrs.
1	0	—	1	0.1	0	
2	3	0.5	2	0.3	1	0.4
3	40	2.1	14	1.1	5	4.5
4	46	3.3	19	1.6	7	5.4
5	23	5.5	26	3.0	3	10.6
6	16	7.5	26	3.6	4	14.2
7	9	7.6	13	5.1	3	17.5
8	5	9.9	3	5.0	1	19.1
9	6	9.2	13	9.2	1	20.3
10	10	9.2	2	10.8	0	
11	7	7.3	6	12.2	0	
12	1	8.4	0	—	0	
13	1	6.3	2	12.1	0	—
14	0	—	1	12.1	1	37.2
15	0	—	1	19.7	0	

The limpets are all collected in July. 1931.

considerably small, while those larger than 17 or 18 mm. in shell-length, the increase of the shell-weight is conspicuously greater.

TABLE V. Relation of shell-weight to shell-length.

Shell length in mm.	Ohshima (1930)		Ohshima (1931)		Mourajima (drier place)		Mourajima (wet place)	
	No.	Ws in dgrs	No.	Ws in dgrs	No.	Ws in dgrs	No.	Ws in dgrs
34.5	0	—	0	—	0	—	0	—
33.5	0	-	0	-	0	-	0	
32.5	1	40.3	0	-	0	—	0	-
31.5	0	—	0	-	0	—	1	37.2
30.5	0		0	—	0	—	0	—
29.5	0	—	0	-	0		0	-
28.5	0		0		0		3	18.5
27.5	5	22.9	0		0	—	1	17.3
26.5	4	21.7	0	—	0	—	5	15.8
25.5	8	17.9	0	—	0		0	—
24.5	19	15.1	0	-	1	19.7	1	12.8
23.5	31	14.0	1	10.7	2	15.5	2	6.1
22.5	39	11.7	3	11.7	2	12.3	2	5.9
21.5	55	9.9	9	10.4	9	10.0	2	5.7
20.5	58	8.7	10	8.7	7	8.9	3	5.6
19.5	38	7.5	22	7.0	10	7.4	2	5.0
18.5	18	6.5	18	6.4	7	5.7	2	3.6
17.5	14	5.0	20	5.0	12	4.5	0	—
16.5	5	4.1	20	4.1	15	3.5	0	—
15.5	0		17	3.1	17	2.7	0	—
14.5	0		21	2.4	12	2.2	0	—
13.5	0	-	15	1.8	11	1.6	0	-
12.5	0		7	1.5	14	1.2	0	—
11.5	0		1	1.4	5	0.9	0	—
10.5	0	—	1	0.7	1	0.7	0	—
9.5	0		4	0.6	0		1	0.5
8.5	0	—	0	—	2	0.3	0	—
7.5	0		0	—	0		0	
6.5	0	-	0		0	-	0	—
5.5	1	0.1	0		0	-	0	—

Ws.—shell-weight.

Comparing the shell-weights of the limpets found in wet places with those found in the drier places, we note at once, that the former is distinctly smaller than the later (see Table V). We may be safe to say that the limpets grow rapidly in length in wet places but slower in shell thickening compared with those found in dry places.

5. *Shell-weight and total-weight* (*body-weight including the shell*) Relation of the total weight to the shell-weight are shown in Table VI and in Fig. 12. For the data of Yunoshima, I am indebted to Prof. E. NOMURA, who kindly permitted me to cite here. He measured from the materials preserved in 70% sea-water alcohol mixed with an equal amount of 3% formalin. While my own measurements were taken from living specimens.

In Table VI, (Wt—Ws) represents body-weight without shell, and (Wt/Ws—1) represents the relation of body-weight to shell-weight. On

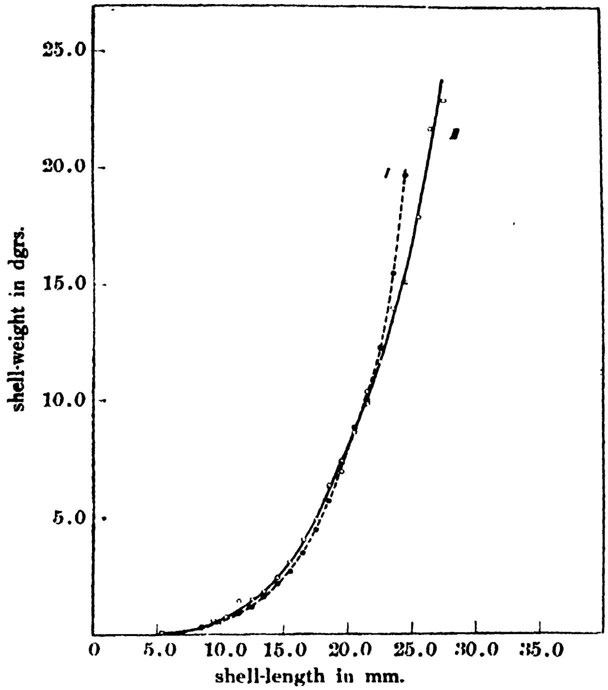

Fig. 11. Growth of shell-weight with respect to shell-length.
I....Mourajima II....Ohshima.

TABLE VI. Total-weight and shell-weight.

Shell-length in mm.	Yunoshima				Ohshima			
	No.	Wt in dgrs	Ws in dgrs	Wt/Ws	No.	Wt in dgrs	Ws in dgrs	Wt/Ws
34.5	1	103.5	49.8	2.08	0	—	—	—
33.5	4	76.7	37.3	2.05	0	—	—	—
32.5	0	—	—	—	1	72.0	40.3	1.78
31.5	2	69.7	35.0	1.99	0	—	—	—
30.5	6	50.6	25.5	1.98	0	—	—	—
29.5	1	52.3	28.7	1.82	0	—	—	—
28.5	5	45.9	23.3	1.97	0	—	—	—
27.5	5	42.6	22.5	1.88	5	40.8	22.9	1.78
26.5	11	31.9	17.0	1.87	4	41.0	21.7	1.89
25.5	27	28.9	16.3	1.78	8	30.8	17.9	1.72
24.5	40	26.0	14.4	1.81	19	27.3	15.1	1.81
23.5	44	23.0	12.9	1.78	31	24.4	14.0	1.74
22.5	51	20.2	11.3	1.79	39	20.9	11.7	1.78
21.5	32	18.0	10.2	1.76	55	17.7	9.9	1.78
20.5	47	15.5	9.6	1.61	58	15.9	8.7	1.82
19.5	38	14.4	8.3	1.72	38	13.7	7.5	1.83
18.5	21	11.3	6.4	1.77	18	9.9	6.5	1.53
17.5	7	9.5	5.3	1.78	14	8.7	5.0	1.74
16.5	4	6.2	3.4	1.82	15	6.7	4.1	1.63

Wt=total-weight, Ws=shell-weight.

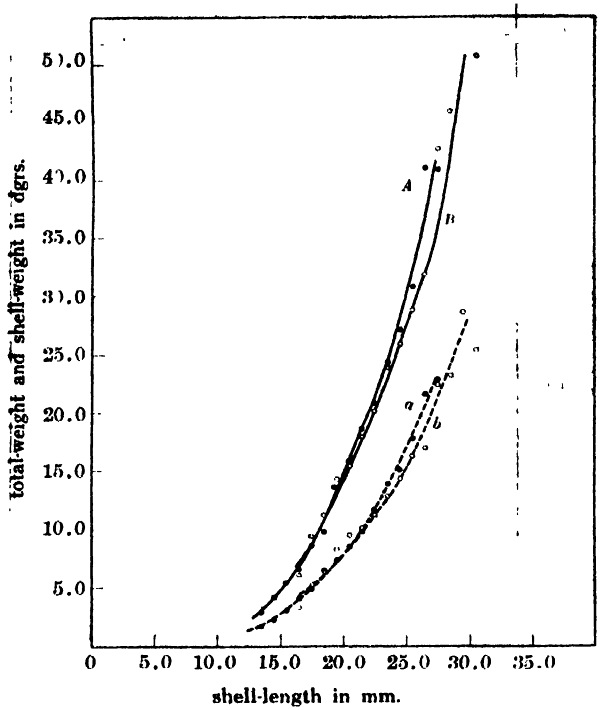

Fig. 12. Total-weight and shell-weight.
A....total-weight (Ohshima) a... shell-weight (Ohshima)
B ...total-weight (Yunoshima) b... shell-weight (Yunoshima)

the limpet of Yunoshima, the average value of Wt/Ws is roughly 1.850 (1.609–1.991) and that of Ohshima is roughly 1.757 (1.598–1.894).

6. *Age and group.* In each colony of *Acmaea dorsuosa* both young and old individuals are found (Fig. 13), and I have determined in each colony the frequencies of limpets with respect to the ages. The results are shown in Table VII.

The frequency distributions shown by 8 different localities are by no means the same but there is no question that the limpets of 3 to 6 years of age are decisively numerous and the frequency decreases as the age increases. Asymmetrical distribution of the frequencies and wider range towards the older than towards the younger seems to suggest that the limpets younger than 3 years do not actively participate in colony forming.

In general, the limpet older than 12 years are few and, as far as I am aware, the limpet of 16 or 17 years old are rarely seen. It seems,

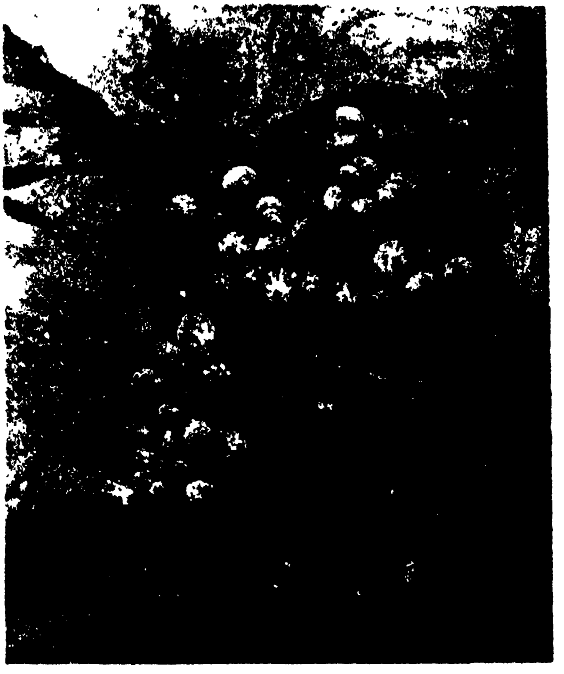

Fig. 13. The colony of *Acmaea dorsuosa*, showing the individuals of different ages.

TABLE VII. Age and group.

Age in years	Ohshima		Mourajima (drier place)			Mourajima (wet place)			Total number
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	
1	0	0	0	0	0	1	0	0	1
2	1	3	6	1	1	1	3	1	16
3	17	38	51	7	9	19	20	2	156
4	47	31	56	8	20	14	29	14	189
5	24	9	21	23	22	34	25	10	148
6	18	8	14	17	27	27	13	20	104
7	9	2	4	7	11	11	3	9	56
8	1	4	3	1	2	6	0	3	20
9	8	5	2	4	6	13	0	2	40
10	14	10	1	2	3	4	0	2	37
11	13	8	0	3	7	3	1	0	36
12	2	0	0	0	1	0	0	0	3
13	1	1	0	0	2	0	0	0	4
14	0	0	0	1	0	0	0	1	2
15	0	0	0	0	1	0	0	0	1
Total number									753

therefore, that the maximum age of *Acmaea dorsuosa* in Mutsu Bay may be about 16 or 17 years.

SUMMARY.

1. The ring method of age determination is applicable to *Acmaea dorsuosa*, GOULD.

2. The growth rate of the limpet which lives in wet places is faster than that of the limpet which lives in drier places, but the former is slower in shell thickening than the later.

3. Relation of the shell-breadth to the shell-length is practically constant with the individuals older than 3 years of age, and the limpet less than 2 years has a rounder shell margin than in the older one in the same colony. In the limpets of equal age, those found in wet places show a rounder shell margin than those found in drier places.

4. The value of (L + B)/2 H decreases with the increase of age, and this is chiefly due to the greater growth in shell-height. In the limpets younger than 4 years of age, the growth rate of shell-height is slower than that of the older one.

When limpets of the same age are compared, those found in wet places have a lower shell-height for given shell-length than those found in drier places, but no difference is found in shell-height between the two.

5. In the limpet, the shell-weight is heavier than the body-weight (without shell), giving the ratio of about 1 : 0.8.

6. The frequency distribution of the age within the colonies is asymmetrical, and the individuals of 4 years of age are most numerous.

7. The maximum age of *Acmaea dorsuosa* in Mutsu Bay may be about 17 years.

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ON THE SEX RATIO AND THE GROWTH OF BODY IN *CARASSIUS AURATUS* AND ITS VARIETY "THE IRON-FISH"[1)]

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(With 6 Text-figures.)

(Received June 2, 1932.)

INTRODUCTION.

Carassius auratus (L.) is one of the most common fresh water fish in Japan and can be obtained very easily in large quantities at any season of year. The well known gold fish is considered to be a variety of *Carassius auratus* (L.) produced under long continued domestication. Recently another natural variety of *Carassius* or the so-called "Iron-fish" was discovered at several localities in the northern part of Honshu.

Prof. SANJI HOZAWA (1927) has published interesting accounts regarding the various external characters of the iron fish contrasted with the characters exhibited by both *Carassius auratus* and gold fish. MATSUI (1931) stated that fishes exhibiting similar external characters as the iron fish are produced artificially from the cross between *Carassius* and "Ryukin" (a kind of a gold fish). TUGE (1929) compared anatomically the principal fiber tracts of the cerebrum among these three fishes. KOBAYASHI (1931) found that these three fishes under consideration exhibit their own characteristic cleaning refrex.

The following observations were made at the Asamushi Marine Biological Station with a view to determine whether or not the iron fish differs from the proper *Carassius auratus* (L.) concerning the sex ratio as well as the growth of body.

During the course of the present research the writer received valuable suggestions from Prof. SHINKISHI HATAI of the Biological Institute of the Tôhoku Imperial University, for which he wishes to acknowledge his deep thanks, and he is also indebted to Prof. SANJI HOZAWA of the Institute for his kind criticisms.

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1) A contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No 88.

MATERIALS AND METHOD.

Most of the fishes employed were caught in the ponds and streams near the Asamushi Marine Biological Station. Those specimens which were used for studying the earlier periods of growth were obtained from three ponds situated on the campus of the station, where *Carassius* and iron-fish were breeding.

As to the measurements of the fish I have adapted the following terms; that is, for total length, the length from the tip of the closed mouth to the extreme end of the caudal fin; for body length, from the tip of the closed mouth to the base of the caudal fin; for depth, the line drawn from both the beginning of the dorsal and ventral fins, were taken. The body of the young fish soon after hatching was measured by means of the reading-microscope. The body weight was determined after removal of surface water and mucus, and the stomach contents as well as of gonads were included.

SPAWNING OF *CARASSIUS AURATUS* AND OF IRON FISH.

The iron fish which were kept in 1931, in the pond No. 1 of this station began to spawn on June 5th and continued for about ten days, while those kept in the pond No. 2, which were the direct off-springs of the fishes in pond No. 1, began to spawn on the 11th of that month. *Carassius auratus* in pond No. 3 began to spawn on June 7th. At the time of breeding, the temperature of water in pond No. 1 was from 15 to 16°C as Table 1 indicates.

TABLE I.

Date	8, A. M.	Midday	4, P. M.
1, June	15.2°C	15.4°C	15.6°C
2, ,,	15.4	15.6	15.6
3, ,,	15.4	15.6	15.6
4, ,,	15.0	15.4	15.4
5, ,,	15.2	16.0	16.0
6, ,,	15.4	15.6	15.8
7, ,,	15.4	15.6	15.6
8, ,,	15.2	15.6	15.6
9, ,,	15.0	15.2	15.0
10, ,,	14.8	15.2	15.2
11, ,,	15.0	15.4	15.2

These eggs began to hatch in about seven days after spawing. In a pond located at Kugurisaka, 2 k.m. from Asamushi, many eggs were found

attached to the leaves of water plants (June 12th), suggesting that the breeding season of *Carassius* and its variety in Aomori Prefecture will be from early June to the middle of that month.

HATCHING RATE AS WELL AS RESISTANCE OF THE IRON-FISH UNDER VARIED CONDITIONS.

1. ***Influence of pH on iron fish eggs.*** In order to test how the H-ion concentration will affect the hatching rate as well as the survival of young fish, 70 eggs of the iron fish in the pond No. 1, were used. These eggs were divided into seven groups of ten each. Each group was placed in a separate basin holding 100 cc. of water but giving different values of pH (3.0 to 8.5). The water temperature varied from 11 to 16.2°C during the course of the experiment. The results are briefly shown in Table 2.

TABLE 2.

No. of eggs	pH	No. of hatch
10	8.5	0
10	7.4	8
10	7.0	8
10	6.7	10
10	5.5	0
10	4.5	0
10	3.0	0

2. ***Influence of sea water on the eggs of iron fish.*** 55 eggs were divided into 11 groups of five each. Each basin contained 100 cc. of water which temperature ranged between 11 and 16.3°C. The results of the test are shown in Table 3.

TABLE 3.

No. of eggs	Medium	pH	No of hatch & remarks
5	normal sea water	8.3	0, died after 24 hours
5	1/2 ,,	8.3	0, died after 2 days
5	1/3 ,,	8.3	3, 2 soon died
5	1/4 ,,	8.2	3, 2 died
5	1/6 ,,	8.2	3, 2 died
5	1/8 ,,	8.2	3, 2 died
5	1/12 ,,	7.9	5, hatched
5	1/16 ,,	7.8	5, hatched
5	1/24 ,,	7.9	4, 1 died
5	1/30 ,,	7.5	5, hatched
5	running water	7.3	5, hatched

3. *Survival of iron fish in sea water.* In this experiment, 45 young iron fishes were divided into 9 groups each, which were subjected to different concentrations of sea water. Each basin contained 100 cc. of variously diluted sea water, the temperature of which was about 16°C. The results are shown in Table 4.

TABLE 4.

No. of fish	Medium	pH	Remarks	pH in 2 days
5	1/2 sea water	8.3	died	8.0
5	1/3 ,,	8.3	4	7.8
5	1/4 ,,	8.3	5	7.8
5	1/6 ,,	8.2	5	7.6
5	1/8 ,,	8.2	5	7.5
5	1/12 ,,	8.0	5	7.4
5	1/16 ,,	7.8	5	7.3
5	1/30 ,,	7.5	5	7.2
5	fresh water	7.2	5	6.9

The results obtained from the above three experiments seem to indicate that, (1) the eggs of iron fish are capable to hatch between pH 5.5 and 7.4, (2) the eggs of the iron fish are capable to hatch normally in sea water only when diluted to 1/12 or more or when the value of pH reaches 7.9, (3) iron fishes are able to survive completely for two days in diluted sea water (1/3 or more).

It is to be regreted that I have made no comparable tests with *Carassius auratus* which contrary to the iron-fish are normally found in brakish water, and one would anticipate a relatively high resistance of the former than in the later to the sea-water.

THE SEX RATIO *CARASSIUS* AND IN ITS VARIETY "IRON-FISH".

1. *Carassius auratus.* SASAKI (1926) has already determined the sex ratio in *Carassius auratus* with the materials obtained in the neighborhood of Sendai and found the ratio 12.6 ♂ : 100 ♀. MATSUI (1930) who examined *Carassius* collected in Toyohashi, Aichi Pref. also obtained nearly identical values of the sex ratio of 13.15 ♂ : 100 ♀. The above observations were made on the adult *Carassius* which were at least over one year old. Recently the present writer examined 700 specimens of *Carassius* of random catch from Numasaki, Aomori Pref., which ranged in length from 5.1 to 29.2 cm. being considerably larger specimens than those examined by SASAKI. The sex ratio of these large fishes was 6 ♂ : 69 ♀

or 0.85 ♂ : 100 ♀. The sex ratio here found can not be readily interpreted owing to the difference in the method of sampling but the result seems to suggest that the sex ratio significantly alters when *Carassius* of larger body sizes alone were examined.

In order to find the sex ratio of *Carassius* in the younger stage, I have collected specimens of about two months after hatching at Tsutsui in the neighborhood of Aomori City on August 15th and on 25th. Only 12 specimens out of 300 collected on the 15th of August showed differentiated gonads and gave the sex ratio of 3 ♂ : 9 ♀, while among those collected on August 25th, 11 specimens out of 186 were determind for the same reason and obtained the sex ratio of 3 ♂ : 8 ♀. The materials examined are small but seem to indicate that the sex ratio in the young stage of *Carassius* is considerably high in favor of the male sex. The body length of younger *Carassius* here examined ranged from 1.8 to 5.7 cm.

2. Iron-fish. On July 19th, 70 young iron fishes were caught by net at Tazawa, Natsudomari Peninsula, Aomori Pref., in which 18 were about forty days old and the others were at least two years or more old. These 48 young fishes were from 2.36 to 4.30 cm. long and the sex ratio was found to be 24 ♂ : 24 ♀. Although these iron fishes were a little over one month old, the differentiation of gonad was more complete than in young *Carassius* of over two months old.

The sex ratios found by the present writer from iron fish, gold fish, and *Carassius* are as follows:

TABLE 5.

		Locality	Sex ratio ♂ : ♀	Remarks
Carassius auratus (L.)		Tsutsui, Aomori	3 : 9	young
		" "	3 : 8	young
		Numasaki, Aomori	6 : 69	adult
Iron-fish		Tazawa, Aomori	24 : 21	young
		" "	6 : 2	adult
		Utorinuma, Miyagi	8 : 7	adult
Gold-fish	wakin	Tokyo	61 : 67	adult
	ryukin	Aomori	53 : 47	adult
	comet	"	24 31	adult

GROWTH OF THE IRON-FISH IN EARLIER PERIOD.

The growth of young iron fish during the first ten days after hatching is shown in Figs. 1 and 2. The materials were obtained from pond No. 1. The characters chosen for measurements were the body depth, the diameter of eye, and the tail length. The rapid reduction of the depth immediately after hatching is due to the absorption of sac, and afterwards it increases slowly, but steadily. The growth of eye in diameter was much faster for the first three days and then slowly as is shown in Fig. 1.

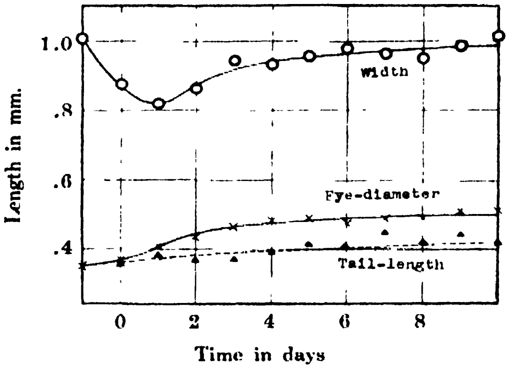

Fig. 1. Mean daily increments of width, eye-diameter and tail-length.

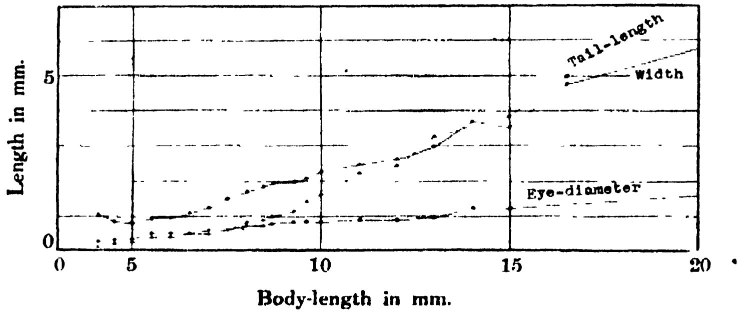

Fig. 2. Showing the relation of growths of width, eye-diameter and tail-length in the young stage of iron fish raised in pond No. 1.

The tail length, at first, is shorter than the diameter of eyes for corresponding body length, but when the body grows to 7.2 mm. in length. (Fig. 2 is based on the data given in Table 7), the length of the former becomes nearly equal to the later, indicating that the tail develops faster than the diameter of eyes and ultimately surpasses the latter, and it even

surpasses the body depth when the fish reaches 120–130 mm. in length. The similar data on the iron fish from the 11th day on after hatching were not obtained for fear that the normal rate of growth would be altered when kept too long in the laboratory.

TABLE 6.

Age in days	Body length mm.	Total length mm.	Depth mm.	Eye-diameter mm.	Tail length mm.	No. of specimens examined
1	–	—	1.01	0.35	—	1
0	5.0	5.4	0.88	0.37	0.37	10
1	5.2	5.5	0.82	0.41	0.39	20
2	5.4	5.7	0.86	0.43	0.38	20
3	5.5	5.9	0.94	0.46	0.38	20
4	5.6	6.0	0.93	0.48	0.40	20
5	5.8	6.2	0.96	0.50	0.43	20
6	5.8	6.2	0.98	0.48	0.42	20
7	5.9	6.4	0.97	0.49	0.46	20
8	6.0	6.4	0.96	0.50	0.44	20
9	6.0	6.5	0.98	0.52	0.45	10
10	6.2	6.6	1.02	0.52	0.43	10

Table 6 shows the growth of different parts of the young iron fish for the period of the first ten days after hatching. The same data when arranged according to the progressive order of the body length are shown in Table 7.

TABLE 7.

Body length mm.	Total length mm.	Depth mm.	Eye diameter mm.	Tail length mm.	B L./Depth	B.L./Tail	No. of specimens examined
3.5	3.6	1.1	0.32	0.1	3.1	25.0	2
3.6	3.8	1.0	0.36	0.2	3.6	20.0	2
4.4	4.7	0.9	0.38	0.3	4.9	16.3	2
4.6	4.9	0.8	0.37	0.3	5.6	15.9	3
4.7	5.0	0.8	0.37	0.3	5.7	13.8	2
4.8	5.1	0.8	0.35	0.3	6.2	15.5	3
4.9	5.3	0.8	0.40	0.4	6.1	13.2	4
5.0	5.4	0.8	0.42	0.4	6.5	13.9	4
5.1	5.5	0.9	0.41	0.4	6.0	13.4	11
5.2	5.6	0.8	0.44	0.4	6.4	14.4	18
5.3	5.7	0.9	0.45	0.4	5.8	14.3	15
5.4	5.8	0.9	0.44	0.4	6.0	13.9	14
5.5	5.9	0.9	0.46	0.4	5.9	13.7	12
5.6	6.0	0.9	0.46	0.4	6.0	13.7	20
5.7	6.1	0.9	0.47	0.4	6.2	13.9	16
5.8	6.2	0.9	0.50	0.4	6.4	14.5	11
5.9	6.3	1.0	0.51	0.4	6.2	14.1	10
6.0	6.4	0.9	0.50	0.4	5.4	14.0	9
6.1	6.5	1.0	0.52	0.4	6.2	14.9	16

Body length mm.	Total length mm.	Depth mm.	Eye diameter mm.	Tail length mm.	B.L./Depth	B.L./Tail	No. of specimens examined
6.2	6.6	1.0	0.52	0.4	6.5	15.1	8
6.3	6.7	1.0	0.54	0.4	6.5	15.4	8
6.4	6.8	1.1	0.52	0.4	5.9	15.6	4
6.5	6.9	1.1	0.53	0.4	5.9	15.9	3
6.6	7.0	1.2	0.53	0.4	5.7	16.1	1
6.7	7.1	1.2	0.53	0.4	5.8	15.2	1
6.9	7.5	1.3	0.59	0.5	5.3	15.3	2
7.0	7.5	1.3	0.60	0.5	5.6	13.5	3
7.1	7.6	1.2	0.58	0.5	5.7	13.7	3
7.2	7.8	1.3	0.64	0.6	5.4	11.8	7
7.3	7.8	1.4	0.62	0.5	5.2	13.8	2
7.4	7.9	1.4	0.59	0.6	5.4	13.5	2
7.6	8.3	1.5	0.68	0.6	5.1	12.7	1
7.7	8.4	1.5	0.69	0.7	5.0	11.9	1
7.8	8.5	1.6	0.69	0.7	4.8	10.5	4
8.1	8.9	1.7	0.73	0.9	4.7	9.4	2
8.3	9.2	1.8	0.68	0.9	4.7	9.3	2
8.4	9.2	1.8	0.72	0.9	4.8	9.1	1
8.5	9.3	1.8	0.72	1.0	4.8	8.9	2
8.6	9.5	1.8	0.74	1.0	4.8	9.0	1
8.7	9.6	1.8	0.75	1.0	4.9	8.9	2
8.8	9.7	1.9	0.77	1.0	4.7	8.9	1
8.9	10.1	1.9	0.74	1.1	4.6	8.2	2
9.2	10.2	2.0	0.80	1.1	4.6	8.2	4
9.3	10.5	2.0	0.76	1.2	4.7	7.8	1
9.5	11.0	2.0	0.80	1.4	4.7	6.9	2
9.7	11.1	2.0	0.74	1.5	4.8	6.6	2
10.0	11.8	2.2	0.83	1.6	4.6	6.1	3
11.0	13.6	2.5	0.86	2.3	4.4	4.7	2
12.0	14.2	2.7	0.91	2.5	4.4	4.9	5
13.0	16.1	3.0	0.96	3.3	4.3	3.9	1
14.0	15.8	3.7	1.23	3.3	3.8		1
15.0	18.9	3.6	1.15	3.9	4.1	3.8	2
16.0	23.0	4.8	–	5.0	3.3	3.2	3

The ratio between the body length and the depth increases at first owing to the absorption of the umbrical sac, which however diminishes at first rapidly and then slowly. This ratio soon approaches to that shown by the adult form which is approximately 3.0 (Table 8). The ratio between the body length and the tail length is 25, immediately after hatching, but it diminishes gradually with the increase of the body length. In 16 mm. in body length, the ratio becomes 3.2, and in 40 mm. it is 2.42 (Table 8), indicating a very rapid increase of the tail which corresponds to that of the body. Indeed, in the iron fish over five years old, the tail length almost equals or even exceeds that of the body, giving the value for the ratio 1 or even less. The relation between body length and depth and the relation between body length and tail length are shown in Table 7 and their graphical presentation in Fig. 3 a.

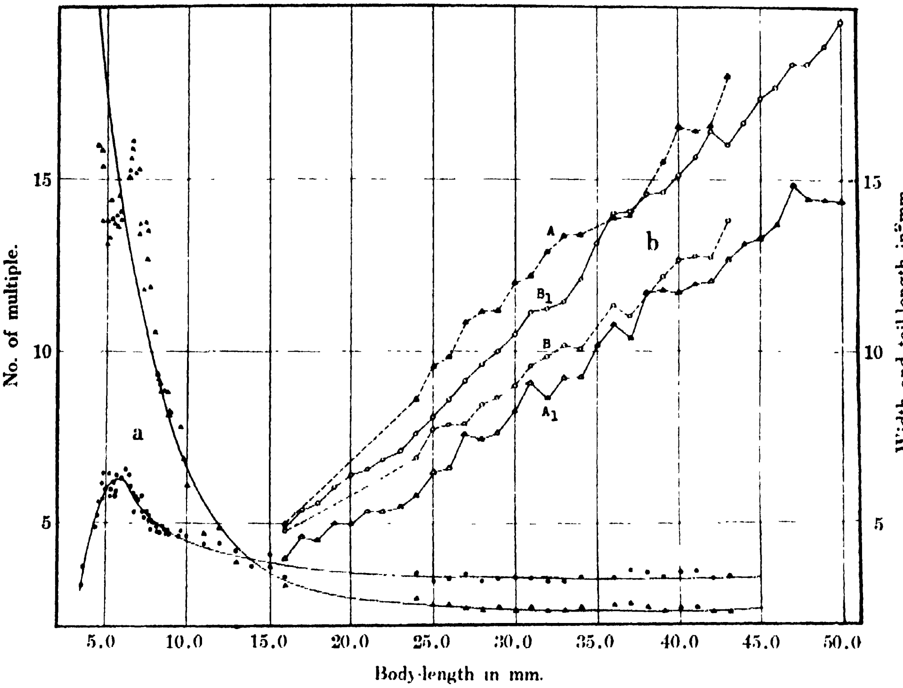

Fig. 3. a Change in the ratio of body-length and tail-length (upper) and in the ratio of body-length and width (lower). b. Showing the difference in tail-length and width between *Carassius* and iron fish. A and A_1 indicate the tail-length of iron fish and *Carassius*; B and B_1 the width of iron fish and *Carassius*, respectively.

BODY MEASUREMENTS TAKEN FROM *CARASSIUS* CONTRASTED WITH THOSE TAKEN FROM IRON-FISH.

One of the most characteristic differences between iron fish and *Carassius* is that the former possesses considerably longer fins and tails than the latter. The writer measured the lengths, weights and depths of the bodies of these two forms and presented the data in Tables 8 and 9.

In Table 8 are shown the data on the measurements taken from young *Carassius* which were found at Tsutsui, Aomori Pref., and the iron fish which hatched in pond No. 1, while in Table 9 are given the data in the adult forms of both *Carassius* and iron fish; *Carassius* here used were obtained from Numasaki, Aomori Pref., and the iron fishes were captured from Tazawa, Aomori Pref. As these data show, the iron fish is lighter in weight, shorter in depth and longer in tail as compared with the respec-

TABLE 8.

B.L. (mm.)	Total L. (mm.)		Total W. (g.)		Depth (mm.)		Tail L. (mm.)		B.L./Depth		B.L./Tail L.	
	Carassius	Iron fish	*Carassius*	Iron fish	*Carassius*	Iron fish	*Carassius*	Iron fish	*Carassius*	Iron fish	*Carassius*	Iron fish
14	17.7	15.8	0.07		4.2	3.7	3.5	-	3.3	3.8	4.0	—
15	-	18.9		—	-	3.6		3.9	—	4.2		3.8
16	20.3	23.0	—	—	4.9	4.8	4.0	5.0	3.3	3.3	4.0	3.2
17	21.6		0.12		5.3	—	4.6	--	3.2	-	3.7	
18	22.1			-	5.6	—	4.0	—	3.2	—	4.5	—
19	24.0	—	0.21		6.1		5.0		3.1		3.8	
20	25.0		0.22		6.4		5.0		3.1		4.0	—
21	26.4	--	0.26		6.6		5.4	-	3.2	-	3.9	-
22	27.4	-	0.31	—	6.8		5.4	—	3.2	--	4.1	
23	28.5	—	0.37		7.1	—	5.5	-	3.2		4.2	—
24	29.8	32.7	0.41	0.34	7.6	6.8	5.8	8.7	3.2	3.5	4.1	2.8
25	31.5	34.6	0.45	0.43	8.2	7.8	6.5	9.6	3.1	3.2	3.9	2.6
26	32.6	35.9	0.52	0.51	8.6	7.9	6.6	9.9	3.0	3.3	3.9	2.6
27	34.6	37.8	0.63	0.56	9.2	7.9	7.6	10.8	2.9	3.4	3.6	2.5
28	35.5	39.2	0.67	0.64	9.6	8.5	7.5	11.2	2.9	3.3	3.7	2.5
29	36.7	40.2	0.74	0.68	10.0	8.7	7.7	11.2	2.9	3.3	3.8	2.6
30	38.3	42.0	0.83	0.78	10.5	9.0	8.3	12.0	2.9	3.3	3.6	2.5
31	40.1	43.2	1.00	0.86	11.1	9.6	9.1	12.2	2.8	3.2	3.4	2.5
32	40.7	44.9	1.10	0.90	11.2	9.9	8.7	12.9	2.9	3.2	3.7	2.5
33	42.3	46.3	1.13	1.07	11.4	10.2	9.3	13.3	2.9	3.2	3.7	2.5
34	43.3	47.3	1.22	1.13	12.1	10.1	9.3	13.3	2.8	3.4	3.7	2.6
35	15.2		1.26	--	13.2	—	10.2		2.7		3.4	—
36	46.8	49.8	-	1.41	14.0	11.4	10.8	13.8	2.6	3.2	3.3	2.6
37	47.4	53.6	1.78	1.34	14.0	11.0	10.4	13.9	2.6	3.4	3.6	2.7
38	49.7		1.72		14.5	-	11.7		2.6		3.3	—
39	50.8	54.5	1.88	1.79	14.6	12.2	11.8	15.5	2.7	3.2	3.3	2.6
40	51.7	60.0	2.07	1.98	15.2	12.7	11.7	16.5	2.6	3.2	3.4	2.4
41	52.9	59.3	2.26	2.14	15.6	12.8	11.9	16.4	2.6	3.2	3.5	2.5
42	54.0	60.4	2.41	2.27	16.4	12.8	12.0	16.5	2.6	3.3	3.5	2.6
43	55.6	61.0	2.46	2.34	16.0	13.9	12.6	18.0	2.8	3.1	3.4	2.4
44	57.1	--	2.71	—	16.6		13.1		2.7		3.4	—
45	58.3		3.04	-	17.4	—	13.3	—	2.6	-	3.4	—
46	59.6		3.24		17.7		13.6		2.6	-	3.4	—
47	61.9		3.57	--	18.4		14.9		2.6	-	3.2	—
48	62.4		3.54		18.4	—	14.4		2.6	—	3.3	--
49	63.3	—	3.77		18.9		14.3		2.6		3.4	—
50	64.4		4.10		19.6		14.4		2.6		3.5	—

tive values of *Carassius auratus*. Consequently, the iron fish has a lower value in the ratio between body length and depth, and higher ratio between body length and tail than those given by *Carassius auratus*. These relations are shown graphically in Fig. 3 b.

TABLE 9.

B.L. (cm.)	Total L. (cm.)		Total W. (g.)		Depth (cm.)		Tail L. (cm.)		B.L./Depth		B.L./Tail	
	Carassius	Iron fish	*Carassius*	Iron fish	*Carassius*	Iron fish	*Carassius*	Iron fish	*Carassius*	Iron fish	*Carassius*	Iron fish
5.0	8.1		4.1	4.5	1.7	1.9	3.1		2.9	2.6	1.6	
5.1	8.5		4.50	6.0	1.6	2.00	3.4		3.2	2.55	1.5	
5.2	8.3		4.90	7.6	1.7	1.90	2.8		3.1	2.74	1.9	
5.3	8.1		4.53	6.4	1.7	1.95	2.8		3.1	2.72	1.9	
5.4	8.3		3.87	5.8	1.7	1.95	2.9		3.2	2.77	1.9	
5.5	8.6		4.50	5.2	1.7	2.03	3.1		3.2	2.71	1.8	
5.6	8.9		4.68	5.9	1.8	2.01	3.1		3.1	2.79	1.8	
5.7	9.2		4.55	6.6	1.8	2.05	3.5		3.2	2.78	1.6	
5.8	8.2		5.34	5.3	1.8	2.12	2.4		3.2	2.74	2.4	
5.9	—		5.71	—	—	2.18	—		—	2.71	—	
6.0	8.8		5.90	7.0	1.9	2.23	2.8		3.2	2.69	2.1	
6.1	9.5		6.16	9.5	2.1	2.26	3.4		2.9	2.70	1.8	
6.2	9.3		6.62	8.9	2.0	2.28	3.1		3.1	2.72	2.0	
6.3	10.0		7.20	8.5	2.1	2.37	3.7		3.0	2.66	1.7	
6.4	9.5		7.53	9.4	2.2	2.40	3.1		2.9	2.67	2.1	
6.5	10.6		7.69	9.7	2.2	2.48	4.1		3.0	2.62	1.6	
6.6	—		7.95	—	—	2.48	—		—	2.66	-	
6.7	10.3		8.58	11.5	2.5	2.54	3.6		2.7	2.61	1.9	
6.8	10.5		9.23	10.0	2.2	2.56	3.7		3.1	2.66	1.8	
6.9	11.2		9.47	11.5	2.2	2.60	4.3		3.1	2.65	1.6	
7.0	10.7		9.29	14.3	2.2	2.64	3.7		3.2	2.66	1.9	
7.1	—		10.05	—	—	2.72	—		—	2.61	—	
7.2	10.7		11.16	10.0	2.4	2.75	3.5		3.0	2.62	2.0	
7.3	—		11.52	—		2.78	—		—	2.63	—	
7.4	11.0		12.07	11.5	2.5	2.82	3.6		3.0	2.62	2.0	
7.5	—		12.42	—	-	2.89	—		—	2.60	—	
7.6	11.2		12.70	12.7	2.4	2.87	3.6		3.2	2.65	2.1	
8.0	12.3		15.58	15.2	2.5	3.05	4.3		3.2	2.62	1.9	
8.2	11.0		16.38	14.0	2.5	3.10	3.8		3.3	2.65	2.2	
8.5	12.1		18.83	17.3	3.6	3.18	3.6		3.1	2.67	2.4	
8.6	12.5		19.74	18.5	2.8	3.30	3.9		3.1	2.61	2.2	
9.2	15.0		24.08	22.0	3.0	3.50	5.8		3.1	2.63	1.6	

THE WEIGHT-LENGTH RELATION OF *CARASSIUS*

As PATON (1898), FULTON (1905) and others have shown already in various kinds of fish, the value of body in weight may be expressed in terms of the body length or $W = a L^3$, in which W represents the body weight, L the body length, and a is constant.

Recently SASAKI (1926) showed that in *Carassius auratus* obtained in the neighborhood of Sendai and from which the gonads were removed, the equation is also applicable or in his case: W grms $= 0.0131$ (L cms.)^3. In the writer's data, the younger groups consisted of 569, which age was about two months old (Table 8), and the older groups consisted of 708, which age was at least one year or more old (Tables 9 and 10), gave

TABLE 10.

B. L. (cm.)	T. W. (cm.)	Depth (cm.)	Cal. No.	B. L. (cm.)	T. W. (g.)	Depth (cm.)	Cal. No.
9.0	23.5	3.4	7	12.0	55.9	4.6	6
9.1	22.4	3.4	4	12.1	58.4	4.6	4
9.2	24.1	3.5	7	12.3	55.4	4.7	4
9.3	25.3	3.5	8	12.4	57.0	4.7	3
9.4	26.0	3.5	8	12.5	62.6	4.7	2
9.5	27.9	3.5	13	12.6	63.5	4.8	6
9.6	26.7	3.6	7	12.7	61.5	4.7	6
9.7	28.7	3.6	8	12.8	65.8	4.8	3
9.8	28.3	3.7	8	12.9	69.1	4.7	3
9.9	30.3	3.7	5	13.1	67.3	4.8	5
10.0	29.8	3.8	8	13.2	70.9	5.0	2
10.1	31.8	3.8	9	13.3	66.8	4.9	3
10.2	32.9	3.8	3	13.5	68.6	5.0	3
10.3	36.1	3.9	5	13.6	78.6	5.0	3
10.4	33.9	3.9	8	13.7	84.3	5.1	3
10.5	36.5	4.0	8	13.9	100.6	5.4	1
10.6	37.4	4.0	10	14.0	82.6	5.2	2
10.7	38.0	4.0	10	14.1	83.1	5.2	2
10.8	38.3	4.0	11	14.2	81.3	5.3	3
10.9	42.1	4.2	8	14.4	97.7	5.6	2
11.0	40.2	4.2	4	14.5	100.6	5.5	1
11.1	42.0	4.2	10	14.6	93.2	5.4	1
11.2	44.5	4.2	7	15.1	103.2	5.7	3
11.3	43.8	4.2	6	15.2	114.7	5.7	2
11.4	48.1	4.4	8	15.9	125.6	6.1	1
11.5	43.4	4.4	7	16.0	133.3	6.1	2
11.6	49.4	4.4	6	16.2	128.8	6.0	2
11.7	53.1	4.6	1	16.5	138.6	6.2	1
11.8	50.7	4.4	3	16.6	128.3	6.2	2
11.9	51.2	4.5	10	17.2	148.4	6.4	1

the equations, W grms $=0.0309$ (L cms.)^3 and W grms $=0.0303$ (L cms.)^3 respectively. The slight difference in the value of the constant here given as compared with that calculated by SASAKI may be due to the difference of locality as well as to the differences of the method, that is, an inclusion of gonads. The results are shown in Figs. 4 and 5. Each circle respresents several observations taken from both sexes. In Fig. 5, females only were used.

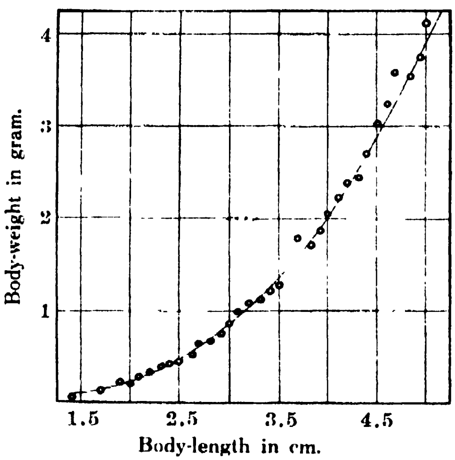

Fig. 4. Showing the relation of weight to body-length in 569 specimens.
($W=0.0309\ L^3$)

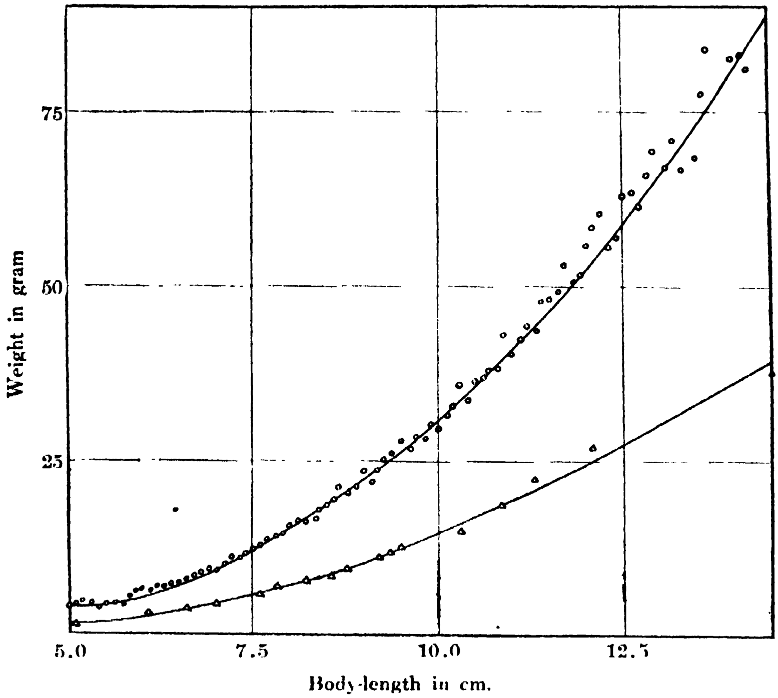

Fig. 5. Showing the relation of weight to body-length in 708 specimens. (*Carassius auratus* L.), the lower figure is from K. SASAKI. All materials are female only.

THE GROWTH OF IRON FISH.

In 1928, about 80 adult iron fishes were placed in one of the ponds of the Asamushi Marine Biological Station, and their external dimensions were measured by the members of the Station at least once every year

TABLE 12.

Age	Body length (cm.)	Tail length (cm.)	Depth (cm.)
Immediately after hatching.	0.56	0.04	0.03
30 Days	3.4	1.3	1.0
70 ,,	4.0	1.6	—
14 Months	6.1	3.2	2.0
26 ,,	8.1	3.9	2.1
37 ,,	8.6	4.3	—
38 ,,	9.4	4.9	2.2
50 ,,	11.4	5.8	2.2

till 1931. The data just mentioned together with the data taken by myself on the young iron fish in 1930 and 1931 are shown in Table 12, and its graphic representation in Fig. 6.

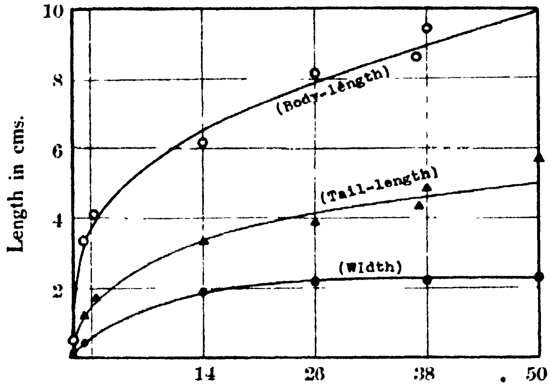

Fig. 6. Growth-curve in iron fish, from birth to 50 months.

From Fig. 6, we can see that the iron fish grows most rapidly in the summer of the first year, and from the second year on, the growth rate is slowed. The fact that the curve takes some upward course even at the age of 50 months, may indicate that the body of fish is capable of growing for longer periods.

THE BODY LENGTH AND DEPTH OF *CARASSIUS*, HAVING IDENTICAL BODY LENGTH BUT AT DIFFERENT AGES.

Of many young *Carassius* which were collected from the river Tsutsui, Aomori, those having the same body length were selected and the depth and the tail length were measured. The results are shown in Table 13. These 11 fishes probably hatched in the spring of 1931. It was found that the fish captured on July 25 possessed a slightly smaller tail length than the fish with identical body length which were captured on August 19, but they were almost equal in depth. Consequently, the growth of the tail region under adverse state is subjected to higher variation than the depth.

A similar comparion was made with the adult *Carassius* which were obtained from various districts. The results show that, in general, the fish which live in pond have a broader body than the fish in river, hence the ratio of body length to depth is smaller in the former than in the

TABLE 13.

Body length	Tail length		Depth	
	July 25.	Aug. 19.	July 25.	Aug. 19.
cm.	cm.	cm.	cm.	cm.
19	0.50	—	0.60	—
20	0.50	0.58	0.64	0.63
21	0.54	0.67	0.66	0.72
22	0.54	0.64	0.68	0.72
23	0.55	0.61	0.71	0.75
24	0.58	0.66	0.76	0.79
25	0.65	0.69	0.82	0.82
26	0.66	0.72	0.86	0.89
27	0.76	0.77	0.92	0.92
28	0.75	0.81	0.96	0.99
29	0.77	0.83	1.00	1.01
30	0.83	0.86	1.05	1.02
31	0.91	0.91	1.11	1.11
32	0.87	0.97	1.12	1.18
33	0.93	1.01	1.14	1.18
34	0.93	1.05	1.21	1.25
35	1.02	1.07	1.32	1.29
36	1.08	1.12	1.40	1.35

later. Such bodily change may be partially due to the relative supply of food substances, being more abundant in the pond and less abundant in the river. In Table 14, are given *Carassius* from the pond Konhei, Asamushi, which are very slender, giving the ratio for between body length and depth as large as 3.02, while in majority of cases, the normal ratio of body length to depth in *Carassius* is in the neighborhood of 2.7 as Table 14 shows.

TABLE 14.

Cal. No.	B.L./Depth	Locality & Remarks	
39	2.45	Bagyu, Miyagi	Pond
3	2.49	Tanuma, Hirosaki	—
28	2.64	Tanabu, Shimokita	--
50	2.65	Numasaki, Aomori	-
4	2.65	Hanatate, Akita	—
5	2.65	Maedaseki, Aomori	—
4	2.70	Kominato, Aomori	—
6	2.73	Iwaki river, Nishigun,	River
4	2.74	Hookuzawa, Kamikita	-
5	2.81	Takahata, Aomori	—
2	2.81	Hirosaki Park, Aomori,	Pond (?)
18	3.02	Konhei, Asamushi	-

WATER CONTENT IN *CARASSIUS*.

As will be anticipated, the percentage of water in younger *Carassius* is very high and diminishes as the body length increases, similar as in other mammalian bodies (HATAI, 1917). The results are shown in Table 15.

TABLE 15.

Fresh body weight	Dry body weight	% of water	Sex and locality
0.33 (g.)	0.045 (g.)	86.30	? Tsutsui
0.53	0.092	82.60	? ,,
0.82	0.152	81.34	? ,,
7.38	1.69	77.10	♀ No. 2.
10.94	2.42	77.88	♂ ,,
22.30	5.28	76.32	♀ ,,
23.10	5.86	74.63	♀ ,,
26.65	6.19	76.77	♂ ,,
55.10	13.67	74.19	♀ ,,
57.11	15.38	73.07	♀ ,,

As will be seen from Table 15, the percentage of water in the male fish is a little higher as against that in the female fish.

SUMMARY.

1. The breeding season of *Carassius auratus* and its variety "Iron-fish" in Aomori Prefecture in 1931 was from early June to the middle of the same month.

2. The sexual differentiation of gonad in *Carassius auratus* becomes distinct in about two months after hatching while that in the iron fish occurs one month earlier.

3. The sex ratio of the iron fish contrary to that of *Carassius auratus* is almost one to one. In this respect the iron fish resembles more to the gold fish.

4. The pH of water influence is striking to both the eggs and young fishes of *Carassius* and of iron fish. Generally, acid is more injurious than alkali.

5. In the iron fish, the annual mean growth is very rapid in the first year and from the next year on the growth rate is gradually diminished.

6. In *Carassius*, the ratio between body length and width is variable, and has a higher value in the fish living in the pond than those living in the river.

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ÜBER DEN ANLAGEPLAN UND DIE KINEMATIK DER FRÜHENTWICKLUNG BEI *HYNOBIUS*.[1]

VON

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(Mit 64 Textabbildungen)

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I. EINLEITUNG.

Die von VOGT ('25) eingeführte örtliche Vitalfärbung hat auf das heiss umstrittene Problem der Frühentwicklung ganz neues Licht geworfen. Die von ihm angegebenen Schemata für die Anordnung der präsumptiven Organanlagen sind heute zur entwicklungsmechanischen Forschung an Amphibienkeimen unentbehrlich.

Im Jahre 1931 führte ich, um die Gestaltungsbewegungen bei der japanischen Urodelengattung *Hynobius* mit denen bei der europäischen zu vergleichen und um ferner den Anlageplan von *Hynobius* festzustellen, Markierungsversuche aus, die das Werden des oberflächlichen Materials des Keims von der jungen Gastrula bis zur Spätneurula verfolgen sollten.

II. MATERIAL UND METHODE.

Als Material benutzte ich Keime von *Hynobius lichenatus* BOULENGER[2] (Syn. *Hynobius unnangso* TAGO) und *Hynobius nigrescens* STEJNEGER (Syn. *Hynobius fuscus* TAGO).

Die Keime wurden nach der von VOGT ('25) angegebenen Methode vital gefärbt. Als Farbstoffe wurden Nilblausulfat und Neutralrot verwendet; kleine Agarstückchen dienten als Farbträger. Die gefärbten

[1] Contributions from the Mt. Hakkoda Botanical Laboratory. No. 15.

[2] In meiner vorigen Arbeit (MOTOMURA '30) nahm ich den Artnamen "*Hynobius unnangso* TAGO", dessen Bestimmung ich Herrn Dr. K. TAGO verdanke, für diese Art an. Im Herbst 1931 sprach Herr Dr T. INUKAI ('32 siehe auch '32 a) auf dem siebenten Kongress der japanischen zoologischen Gesellschaft zu Kyoto die Meinung aus, dass *Hynobius unnangso* TAGO ein Synonym für *H. lichenatus* BOULENGER und *H. fuscus* für *H. nigrescens* STEJNEGER sei. Ich weiss nicht, was davon zutrifft Aber ich will später noch die älteren Namen benutzen, bis diese nomenklatorische Frage völlig geregelt ist.

Agarstückchen wurden etwa 45 Minuten lang beim Keim verwendet. Bei dieser Dauer hielten sich die Farbmarken am längsten, ohne die Entwicklung merklich zu schädigen. Die markierten Embryonen zerlegte ich in frischem Zustand mit einer feinen Glasnadel und Haarschlinge.

III. EXPERIMENTELLER TEIL.

Experiment Nr. I.

L. 720. Acht abwechselnd rote und blaue Marken wurden an einem *Lichenatus*-Keim im Stadium des Gastrulationsbeginns in der Weise angebracht, dass sie die mit dem Äquator parallel liegende Zone in der oberen Halbkugel bezeichneten (Abb. 1 u. 2). Die Invaginationsgrube trat etwa bei 50° unter dem Äquator auf. Nach 23 Stunden hatte der Keim einen ringförmigen Urmund. Alle Marken dehnten sich gleichmässig in der Richtung der Meridiane aus und griffen in die untere Halbkugel ein (Abb. 3). Die Furchungshöhle zeigte sich als ein Hügel des Ektoderms am Antipol der dorsalen Urmundlippe. Nach 43 Stunden hatte der Keim einen kleinen Dotterpfropf. Alle acht Marken lagen allseitig der Urmundlippe an und standen radial zum Dotterpfropf (Abb. 4 u. 5). Nach 65 Stunden war der Keim im Anfang des Neurulationsstadiums (Abb. 6 u. 7). Der Dotterpfropf war zum grossen Teil aufgesaugt worden. Die acht Marken standen radial an der kleinen Urmundöffnung, ganz wie im vorigen Stadium. Aber sie näherten sich der dorsalen Mediallinie infolge der dorsalen Konvergenz und der ventralen Divergenz des präsumptiven Ektoderms in dem von VOGT definierten Sinne so sehr, dass die vier dorsalen Marken in die Medullarplatte aufgenommen wurden, die vier übrigen Marken hingegen die ganze hintere Oberfläche der präsumptiven Epidermis einhüllten. Die Marken c und h befanden sich am inneren Rand der seitlichen Medullarwürste.

Das vordere Ende der dorsalen Marken erreichte nicht den vorderen Teil der Medullarwürste. Die langgestreckten Farbmarken lagen parallel zur Längsachse des Keims. Sie waren annähernd gleich lang wie der oberflächliche Teil des Keims. In diesem Stadium wurde der Keim durch dorsalen Medianschnitt bis zur Urdarmhöhle geöffnet. An der vorderen Medullarwürste wurde das Dachmaterial des Keims Y-förmig beiderseits antero-lateral eingeschnitten. Dann wurde die drei durch Einschnitte hergestellten Lappen des Urdarmdachs aufgeklappt, um die innere Seite des Keims zu beobachten. An dem inneren Rand der Urmundlippe stiessen die acht regelmässig angeordneten Farbmarken an die in der Keimober-

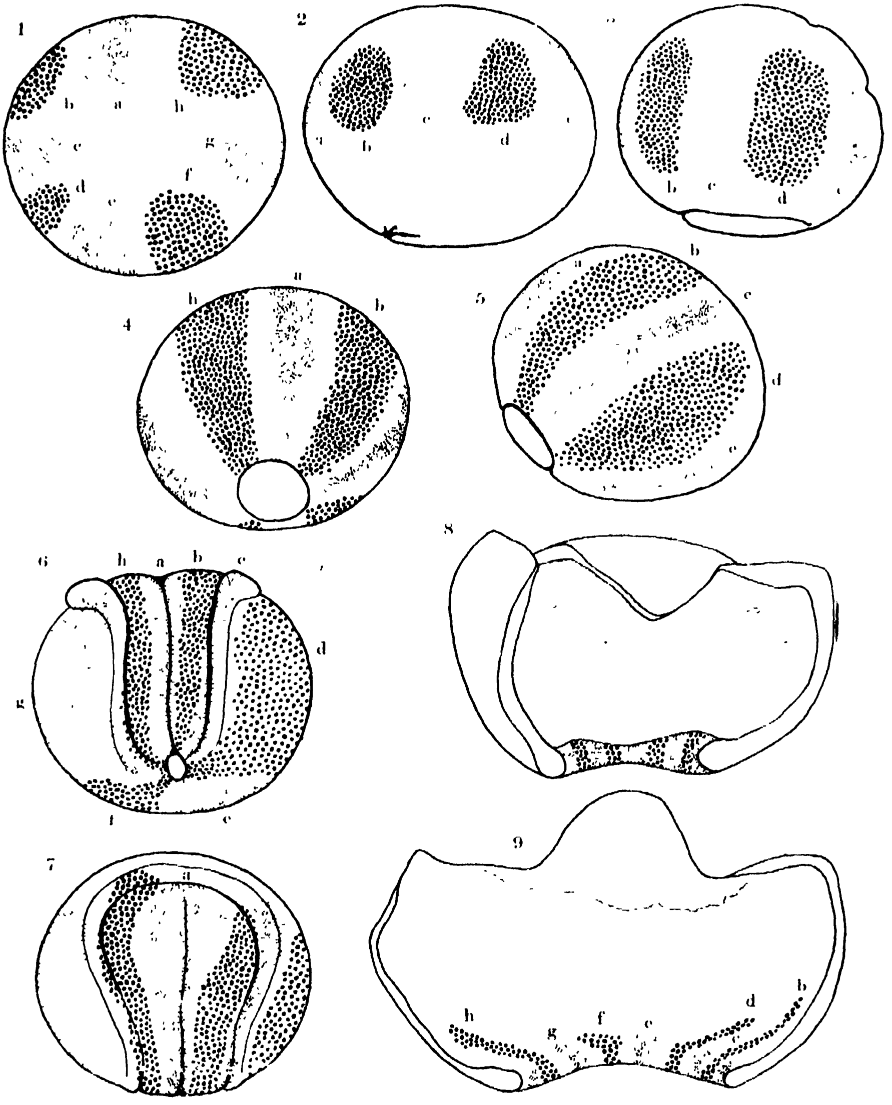

Abb. 1–9. *L. 720.* Markierung der oberen Teil des Äquators einer jungen Gastrula. 1: —Polansicht kurz nach dar Markierung. 2: Gastrula im Profil von rechts kurz nach Markierung. 3: —nach 23 Stunden Profilansicht von rechts. 4 und 5: nach 43 Stunden. 6 und 7: —nach 65 Stunden. 8: —Eröffnung des Urdarms durch dorsalen Medianschnitt. 9: —weitere Präparation. Freilegung der visceralen Mesodermfläche durch Entfernung des Entoderms.

fläche befindlichen Marken an und lagen anderseits unter der Dottermasse des Urdarmbodens verborgen (Abb. 8). Nach der Entfernung der Dottermasse sah man, dass die inneren Ränder der Marken nur ein wenig in das Mesoderm übergegangen waren (Abb. 9). Und sie waren weit hinter den vorderen freien Rand des Mesoderms gerückt. Hier beobachtete ich auch die dorsale Konvergenz und die ventrale Divergenz des umgeschlagenen Materials.

Aus diesem Experiment erkennt man die präsumptive Lage des Ektoderms. Es liegt in der oberen Halbkugel des jungen Gastrulakeims. Die untere Grenze des Ektoderms nähert sich den unteren Rändern der Farbmarken, bleibt aber ein wenig höher als diese. Denn aus dem unteren Teil der Farbmarken muss ein kleiner Teil, der in die innere Seite des Keims umgeschlagen ist, herausgezogen werden.

Auch im Stadium der Anfangsgastrula ist die präsumptive Einstülpungsgrenze an der dorsalen Seite ein wenig höher als an der ventralen. Daher lässt sich aus vielen gleichartigen Experimenten vermuten, dass die präsumptive Einstülpungsgrenze der beginnenden Gastrula an der dorsalen Seite etwa 15° und an der ventralen Seite etwa 10° über dem Äquator liegt.

Im Stadium der Neurulation wurden immer vier von den acht Marken auf die Medullarplatte aufgenommen. Daher entspricht die Grenzlinie zwischen der präsumptiven Epidermis und der Medullarplatte dem seitlichen Meridian der jungen Gastrula, wenigstens im unteren Teil des präsumptiven Ektoderms. Alle Farbmarken lagerten sich in allen Stadien dieses Experiments immer meridional zur Vertikalachse der jungen Gastrula. Selbst im Verlauf der Medullarplattenbildung wurden diese Verhältnisse nicht verändert. Das zeigt, dass die Medullarplatte nicht durch die Konkrescenz der beiden halben Teile sondern durch die dorsale Konvergenz und die ventrale Divergenz der präsumptiven Epidermis gebildet wird.

Experiment Nr. II.

L. 715. An einem *Lichenatus*-Keim wurden acht, abwechselnd rote und blaue Farbmarken am Äquator und eine blaue Marke am oberen Pol im Gastrulationsbeginn angebracht (Abb. 10 u. 11). Nach 24 Stunden hatte der Keim einen ringförmigen Urmund. Alle Äquatormarken reichten allseitig an die Urmundlippe und stand radial zum Dotterpfropf (Abb. 12). Aber die blaue Polmarke war noch in diesem Stadium am oberen Pol geblieben, von der Furchungshöhle nicht betroffen. Daher ist die dorsale Strecke von der Polmarke bis zu der dorsalen Urmundlippe kleiner als

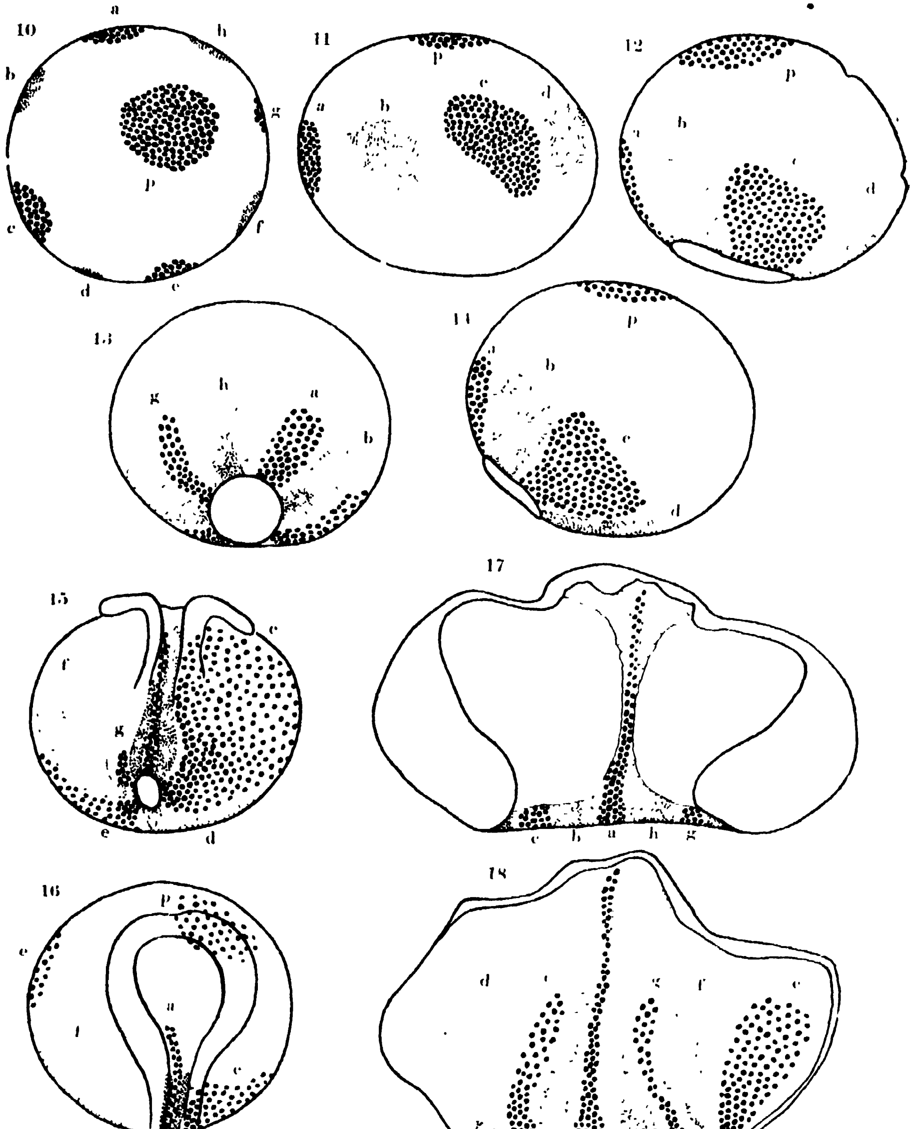

Abb. 10–18. *L. 715* Markierung des Äquators und des oberen Pols einer jungen Gastrula. 10:—Polansicht des Anfangsstadiums. 11:—Profilansicht des Anfangsstadiums. 12:—Nach 24 Stunden. 13 und 14:—Nach 43 Stunden. 15 und 16:—Nach 72 Stunden. 17:—Eröffnung des Urdarms in Rückenlage durch ventralen Medianschnitt. 18: —Weitere Präparation. Freilegung der Chordaanlage und der visceralen Mesodermfläche durch Entfernung des Entoderms

die ventrale Strecke bis zur ventralen Urmundlippe; d.h. die erste misst 120° und die letzte 180°. Der Durchmesser des Dotterpfropfs misst etwa 60°. Nach 43 Stunden wird der Dotterpfropf kleiner. Er misst 35° im Durchmesser (Abb. 13 u. 14). Die Äquatormarken zeigten keine bemerkenswerte Veränderung, ausser dass sie infolge fortschreitender Einrollung am Urmundrand nur schmaler als im vorigen Stadium wurden. Die dorsale Strecke von der Polmarke bis zur dorsalen Urmundlippe misst 120° und die ventrale Strecke bis zur ventralen Urmundlippe 205°.

Nach 72 Stunden war der Keim im Anfang der Neurulation. Der Dotterpfropf war zum grössten Teil aufgesaugt (Abb. 15 u. 16). Die oberen Reste der Äquatormarken lagerten sich radial um die kleine Urmundöffnung. Sie bedeckten bis zur halben Länge die Medullarplatte und neigten auch stark zu dorsaler Konvergenz und ventraler Divergenz. Die Polmarke lag am vorderen Ende der Medullarwürste.

Nach 73 Stunden wurde der Keim in Rückenlage durch ventralen Medianschnitt bis an die Urdarmhöhle eröffnet; dann wurde am vorderen Ende des Medianschnitts, von der vorderen Medullarwürste ausgehend, beiderseits ein Y-förmiger Einschnitt gemacht, um die innere Seite der Urdarmhöhle zu beobachten. An der inneren Seite der Urmundlippe wurden acht, abwechselnd rote und blaue Farbmarken, die den unteren, ungeschlagenen Teilen der Äquatormarken entsprechen, beobachtet (Abb. 17). Und diese innen im Keim sich lagernden Farbmarken sind in ihren vorderen Teilen mit Entoderm bedeckt, mit Ausnahme der dorsalen Mediallinie des Urdarmdachs, wo die Anlage zur Chorda liegt und die darauf liegende blaue Marke (a) zwischen den Nähten der beiderseitigen Entodermränder entblösst ist. Am vorderen Teil der Chordaanlage bogen die beiderseitigen Entodermnähte von der Mediallinie ab. Dieses Gebiet ist die prächordale Platte. Hier traten nicht nur die mediane blaue Marke, sondern auch die noch seitlich liegenden roten Marken, die in den Abbildungen mit b und h bezeichnet sind, auf der inneren Oberfläche der Urdarmhöhle hervor. Es war mir nicht möglich, die vorderen Teile der Entodermnähte noch weiter nach vorn hin zu verfolgen. Aber auf Grund vielfacher Beobachtungen halte ich es für möglich, dass das vordere Ende um das Gebiet der prächordalen Platte verschwindet und der vordere Rand des Mesoderms hier endet.

Durch Entfernung des Entoderms wurden alle Farbmarken an der visceralen Mesodermfläche herauspräpariert. Alle an der Urmundlippe liegenden Farbmarken setzten sich in den kranio-kaudalen Farbstreifen im Mesoderm fort (Abb. 18). Die drei dorsalen Marken a, b und h

hatten das vordere Ende erreicht, aber die fünf übrigen ventralen waren noch in der Körpermitte geblieben. Eine starke dorsale Konvergenz im Gebiete der Körpermitte wurde in den fünf dorsalen Marken beobachtet (Abb. a, b, c, h und g). Die Erweiterung der vorderen Enden der drei übrigen ventralen Marken (d, e und f) ist das Zeichen ventraler Divergenz.

L. 718. An einem *Lichenatus*-Keim wurden acht Äquatormarken und eine Polmarke wie beim vorigen Exemplar im Gastrulationsbeginn ange-

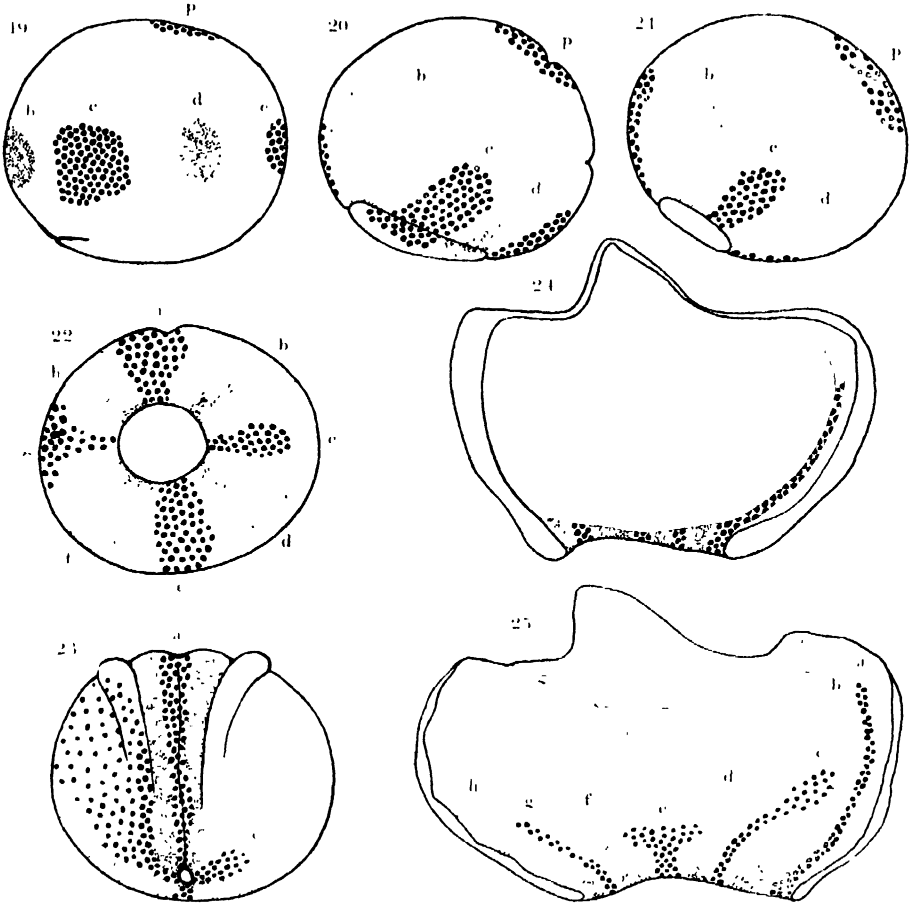

Abb. 19–25. *L. 718.* Markierung des Äquators und des oberen Pols. 19 und 20: — Anfangsstadium. 21: — Nach 24 Stunden. 22: — Nach 43 Stunden. 23: — Nach 71 Stunden. 24: — Eröffnung des Urdarms des Neurulakeims durch linken dorsalen Paramedianschnitt 25: Freilegung der Chordaanlage und der visceralen Mesodermfläche.

bracht (Abb. 19). Die Entwicklungsvorgänge waren ganz gleich wie beim Keim *L. 715*. Nach 71 Stunden wurde bei beginnender Neurula der Keim durch einen dorsalen paramedianen Schnitt auf der linken Hälfte der Medullarplatte eröffnet und am Vorderende der Medullarplatte in kraniolateraler Richtung beiderseits Y-förmig sorgfältig eingeschnitten. Dann wurden die drei durch die Einschnitte hergestellten Lappen des Urdarmdachs sorgfältig aufgeklappt. Kleine Teile der umgeschlagenen Äquatormarken wurden an der inneren Seite der Blastoporuswürste beobachtet (Abb. 24). Aber die meisten vorderen Teile waren zwischen das Ektoderm und das Entoderm eingedrungen. Nur die Chordaanlage zwischen den Entodermnähten des Urdarmdachs war entblösst. Nach Entfernung des Entoderms und der Dottermasse wurde die viscerale Seite des Mesoderms sichtbar (Abb. 35). Die Äquatormarken setzten sich in dem Mesoderm in longitudinalen Farbstreifen fort, die vorher durch das Entoderm verdeckt waren. Die dorsalen Farbstreifen sind länger als die ventralen. Auf der ventralen Seite reichen die Farbmarken nur bis zur Mitte des Mesoderms. Der vordere Teil des Mesoderms blieb ungefärbt. Aber in den gefärbten Teilen des Mesoderms traten die typische dorsale Konvergenz und die ventrale Divergenz deutlich hervor. Der vordere Rand des Mesoderms zeigte unregelmässige Kontur.

Aus den Keimen *L. 715* und *L. 718* wurde die Grenze der Einrollung festgestellt. Sie liegt annähernd parallel zum Äquator, aber etwas höher als dieser. Durch den Vergleich derselben Experimente erkannte ich, dass die präsumptive Lage der Einstülpungsgrenze in beiden Fällen übereinstimmend auf der dorsalen Seite etwa 15° über dem Äquator und auf der ventralen Seite 10° über dem Äquator ist.

Die untere Grenze des Mesoderms liess sich aus dem Experiment nicht feststellen. Im Verlauf der Gastrulation dringen die markierten Teile der Mesodermanlage, die sich anfangs an der Randzone gelagert hatten, durch Einrollung in das Innere zwischen Ektoderm und Entoderm ein. Und die Chordaanlage verhält sich auch wie das Mesoderm, eben bis zu diesem Neurulastadium. Die Richtung der Einrollung ist immer longitudinal zur Körperachse, oder mit anderen Worten: senkrecht zur Urmundlippe. In der Körpermitte drängen sich die Marken an der dorsalen Mediallinie zusammen. Deswegen verbreiten sich die ventralen Mesodermmarken nur ganz dünn, d.h. die dorsale Konvergenz und die ventrale Divergenz zeigen sich auch im Mesoderm.

Das Entoderm klebt am Mesoderm fest. Und nur an der inneren Seite der Urmundlippe und der Chordaanlage fehlt es an Entodermfütte-

rung, d.h. bis zum Neurulastadium. Aber das Entoderm selbst konnte mit einer feinen Glasnadel entlang den beiderseits der Chordaanlage liegenden, dorsalen Nähten von dem sich eng anlegenden Mesoderm ganz frei und leicht abgetrennt werden. Aber die Grenzlinie zwischen Entoderm und prächordaler Platte ist hier nicht so deutlich bei der Entfernung des Entoderms, weil das erste allmählich in die zweite übergeht.

Im Verlauf der Gastrulation lagert sich das Material des oberen Pols näher an der dorsalen Urmundlippe als an der ventralen. Aber nach dem Erscheinen der Medullarplatte befindet sich die Polmarke an vorderen Ende der Medullarwürste und an der davor liegenden Kopfhautanlage. Danach lässt sich vermuten, dass der obere Pol der jüngsten Gastrula die Grenze zwischen dem vorderen Ende der Medullaranlage und der Kopfhautanlage ist.

Experiment Nr. III.

N. 203. Eine Skala von fünf abwechselnd roten und blauen Marken wurde an einem Nigrescens-Keim im jüngsten Gastrulastadium über den seitlichen Meridian hin angebracht (Abb. 26). Nach 24 Stunden hatte der Keim einen ringförmigen Urmund. Eine blaue und eine rote Marke rollten sich ins Innere des Blastoporus ein, und ein Teil der mittleren blauen Marke blieb noch am seitlichen Rand der Blastoporuslippe übrig (Abb. 27 u. 28). Der untere Teil der Skala wendete sich nach der dorsalen Seite der Urmundlippe. Nach 42 Stunden befand sich der Keim im beginnenden Neurulastadium. Die oberste blaue Marke lagerte sich am seitlichen Rand der Medullarplatte (Abb. 29 u. 30). Nach 48 Stunden hatte sich die Medullarplatte völlig gebildet. Die auf der Oberfläche gebliebenen blauen und roten Marken lagerten sich hintereinander an dem kaudalen Teil der Medullarwürste (Abb. 31, 32 u. 33). In diesem Stadium wurde der Keim in der dorsalen Mediallinie aufgeschnitten und am vorderen Ende der Medullarplatte links so, dass sich der linke Teil der Medullarplatte mitsamt dem Urdarmdach links aufklappen liess. Dann ward der Keim sorgfältig eröffnet (Abb. 34).

An der inneren Seite der Blastoporuswürste beobachtete ich eine kurze blaue Marke, die der obere Teil der mittleren Marke c ist. An der linken Seite des Urdarmbodens befand sich eine blaue und eine rote Marke, die die unteren Marken der Abb. 26 identisch sind.

Der Keim wurde nochmals an der linken Seite des Urdarmbodens entlang der in Abb. 34 gezeigten punktierten Linie eröffnet. Die Dottermasse des Urdarmbodens wurde von dem Mesoderm abgetrennt (Abb. 35).

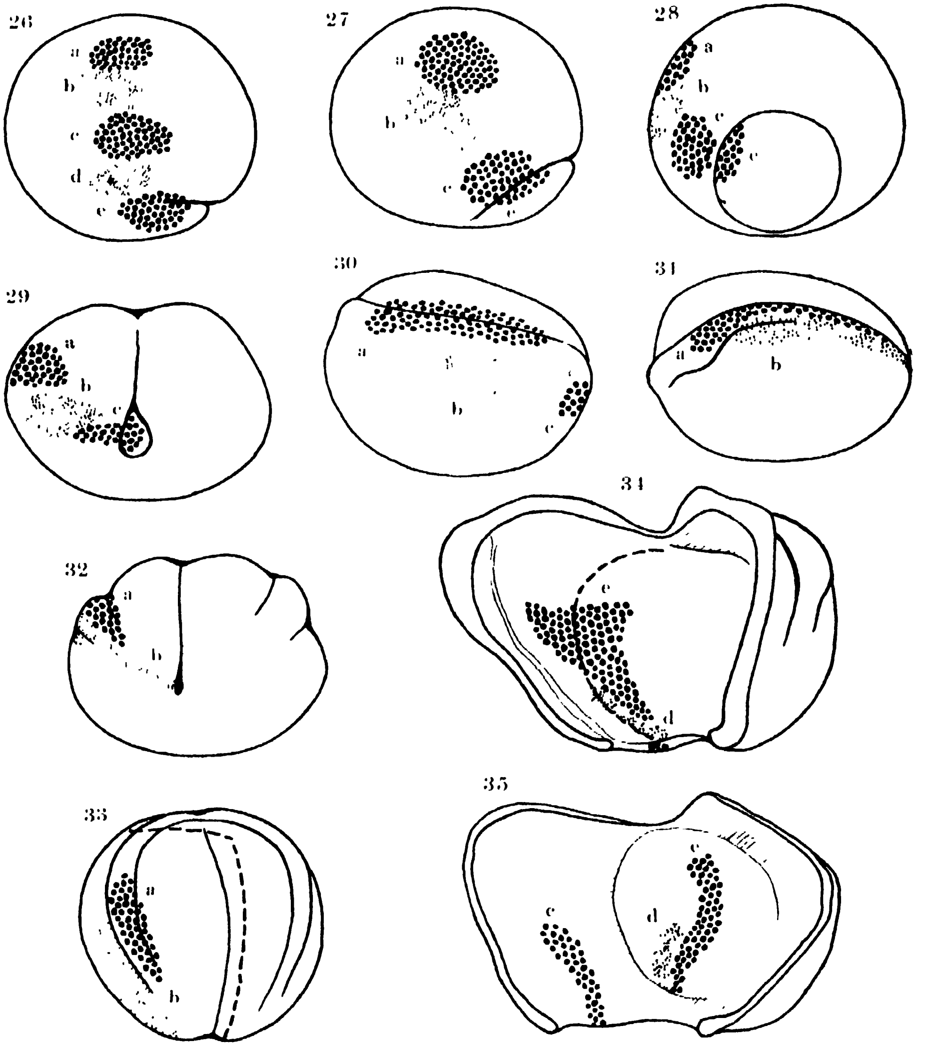

Abb. 26–35. *N. 203.* Lateral-meridionale Markierung der jungen Gastrula. 26: — Anfangsstadium. 27 und 28: — Nach 24 Stunden. 29 und 30: — Nach 42 Stunden. 31–35: — Nach 48 Stunden. 34: — Eröffnung des Urdarms durch rechten dorsalen Paramedianschnitt. 35: Freilegung der lateralen visceralen Mesodermfläche durch Anhebung des Entoderms.

Die mittlere blaue Marke c in der Abb. 34 hatte sich in longitudinalen Farbstreifen im seitlichen Mesoderm fortgesetzt, erreichte aber nicht das

vordere Ende des seitlichen Mesoderms.

Aus diesem Experiment wurde der Anlageplan des seitlichen Keimbezirks klar. Die Marken a und b lagerten sich in dem Ektoderm an der Grenze zwischen Medullarplatte und Hautektoderm. Die Marke c lag ausschliesslich im Mesoderm. Die Marken d und e lagerten sich seitlich an der Wand des Urdarmbodens. Sie sind also im Entoderm. Die Marke e neigte zu einer eigentümlichen Gestaltungsbewegung und wendete sich in kranio-dorsaler Richtung nach dem seitlichen Urdarmboden. Diese Bewegung verwickelte sich natürlich in die Bildung des Darmkanals. Die Gestaltungsbewegung des Entoderms wird im Folgenden eingehend analysiert.

Experiment Nr. IV.

N. 201. Eine Skala von fünf abwechselnd roten und blauen Marken wurde an einem *Nigrescens*-Keim im jüngsten Gastrulastadium von der dorsalen Urmundlippe bis zum ventralen Äquator auf der Mittellinie der unteren Keimhälfte angebracht (Abb. 36). Abb. 37 zeigt die untere Polansicht kurz nach der Markierung. Etwa 22 Stunden nach der Markierung hat der Keim hufeisenförmigen Urmund. Die Hälfte des dorsalen Teils der Skala ist schon von der dorsalen Urmundlippe bedeckt (Abb. 38). Nach 46 Stunden erreicht der Keim bereits das Stadium der jüngen Neurula. Ein Rest der letzten blauen Marke blieb am ventralen Rand des geschlossenen Urmunds (Abb. 39). In diesem Stadium wurde der Keim durch dorsale Schnitte wie beim Keim des Experiments Nr. I eröffnet (Abb. 40).

Die vier Farbmarken lagen in den hinteren zwei Dritteln des Urdarmbodens richtig geordnet. Die blaue Marke, die sich anfangs am ventralen Äquator befunden hatte, kam jetzt an den ventralen Urmundrand. Die Teile der Marken d und e waren natürlich in das ventrale Mesoderm umgeschlagen. Die vorderste Marke, die sich im Anfang dicht hinter der dorsalen Urmundlippe befand, verlängerte sich sehr merkwürdig gegen die seitlichen Wände des Urdarms hin. Auch die ihr folgenden Marken zeigten die gleiche Tendenz, obwohl sie in den hinteren Marken nicht deutlicher ist. Noch eine hellblaue Marke wurde vor der vordersten blauen Mark a gefunden. Sie wurde niemals am Anfang planmässig angelegt, sondern vielleicht zufällig bei der Einrollung der dorsalen Urmundlippe von der vordersten blauen Marke a auf das darüber gelagerte Material der dorsalen Urmundlippe eingedrückt. Diese hellblaue Marke verlängerte sich auch lateralwärts sehr deutlich. Der ungefärbte, zwischen der hell- und dunkel-

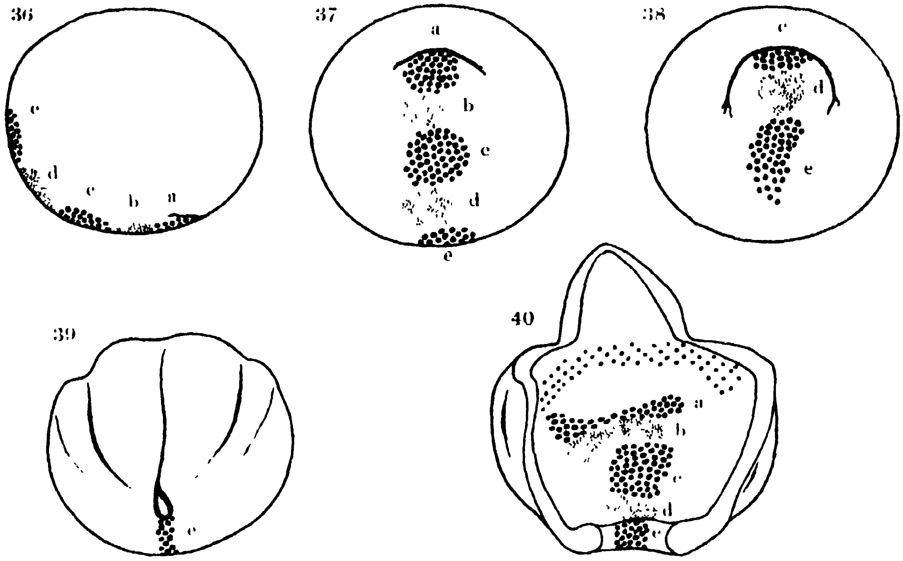

Abb. 36-40. *N. 201.* Mediane Markierung auf dem Dotterfeld der jungen Gastrula. 36:—Anfangsstadium im Profil von links. 37:—Anfangsstadium. Untere Polansicht. 38:—Nach 22 Stunden 39:—Nach 46 Stunden. 40. Eröffnung des Urdarms durch Medianschnitt.

blauen Marke liegende Teil des vorderen Darmbodens ist vermutlich die Keimoberfläche, die sich wohl im frühesten Gastrulastadium in der Nähe des Urmundes gelagert hat und in der Zeit der Markierung durch den Einrollungsvorgang schon in die Blastoporen eingedrungen ist.

Aus diesem Experiment liess sich Folgendes feststellen. An der ventralen Mediallinie ist die Grenze der Einrollung nicht höher als das obere Ende der blauen Marke e, d.h. etwa 10° über dem Äquator der ventralen Seite. Und die präsumptive Grenze zwischen dem Dotterentoderm und dem ventralen Mesoderm liegt in der Mitte der roten Marke d, d.h. etwa 20° unter dem Äquator. Die Bewegungsrichtung des präsumptiven Entoderms ist ganz verschieden von der des Ektoderms und des Mesoderms. Wir erkannten schon aus obigen Experimenten, dass sich das präsumptive Ektoderm ebenso wie das Mesoderm bei der Gastrulation in meridionaler Richtung verlängert. So ist es nicht beim Entoderm. Das Material des präsumptiven Urdarmbodens verlängert sich senkrecht zur Mediane des Keims. Diese Tendenz ist bemerkenswert deutlich an dem im frühesten Gastrulastadium sich einrollenden Teil, der sich im Medullarplattenstadium am vorderen Drittel des Urdarmbodens im Kopfdarmboden lagert. Die

Gestaltungsbewegung der seitlichen Urdarmwand wurde im folgenden Experiment festgestellt.

Experiment Nr. V.

N. 210. Sechs abwechselnd rote und blaue Marken wurden an einem *Nigrescens*-Keim im frühen Gastrulastadium sechseckig rings um die dorsale Urmundlippe so angelegt, dass eine blaue auf die dorsale Urmundlippe kommt, zwei rote sich beiderseits von dieser blauen Marke lagern und die noch eine übrigbleibende rote Marke in das Dotterfeld auf dem vegetativen Pol zu liegen kommt, so dass die übrigen zwei blauen Marken symmetrisch zu beiden Seiten der letztgenannten roten Marke stehen (Abb. 41).

Etwa 22 Stunden nach der Markierung hatte der Keim einen hufeisenförmigen Urmund. Die erste blaue und die anfänglich erwähnten zwei roten Marken waren unter die Urmundlippe eingerollt. Und die drei Marken, die am vegetativen Pol waren, blieben auf dem Dotterpfropf übrig

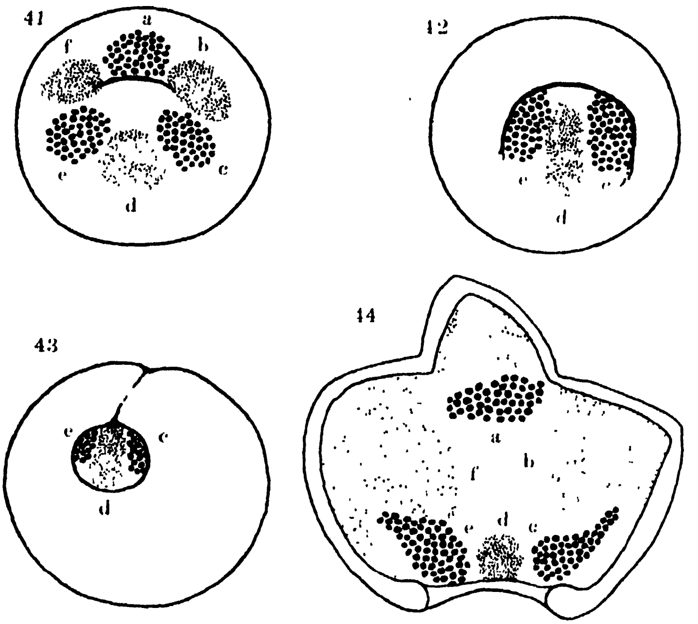

Abb. 41–44 *N. 210.* Markierung des Entodermbereichs. 41:—Anfangsstadium. 42:—Nach 22 Stunden. 43 Nach 48 Stunden. 44:—Eröffnung des Urdarms im Neurulastadium durch dorsalen Medianschnitt.

(Abb. 42). Etwa 48 Stunden nach der Markierung befand sich der Keim im frühen Neurulastadium. Alle Marken waren im Keim verborgen. In

diesem Stadium wurde der Keim durch dorsalen Schnitt wie gewöhnlich eröffnet Abb. 44). Die rote Marke, die sich anfangs am vegetativen Pol befand, war jetzt am hinteren Rand des Urdarmbodens. Die beiden blauen Marken, die sich beiderseits der eben genannten roten Marke lagerten, befand sich in der Nähe der roten Polmarke und hatten sich in dorsolateraler Richtung bis an die dorsalen Entodermnähte verlängert.

Die blaue Marke, die anfangs auf der dorsalen Urmundlippe war, befand sich am vorderen Ende des Urdarmbodens und zeigte eine merkwürdige seitliche Verlängerung, ganz wie beim Versuch Nr. IV.

Die beiden anfangs in der Nähe der blauen Marke der Urmundlippe befindlichen roten Marken zeigten grosse Ausdehnung. Sie erreichten die Entodermränder der dorsalen Entodermnähte und umgaben den vorderen Teil der vorderen blauen Marke, mit Ausnahme der vorderen Mediane des Kopfdarms, wo das Material entweder rot oder blau gefärbt war. Hier lag die sogenannte prächordale Platte. Die Ausdehnung dieser roten Marken ist drei- oder vierfach grösser als die der blauen Marke. Die Richtung der Erweiterung dieser roten Marken ist vermutlich seitlich und kranio-lateral.

Dieser Versuch zeigt, dass die meisten Teile der Urdarmwand aus dem Material des durch die sechs Marken markierten Bereichs gebildet werden. Die Gestaltungsbewegungen des Entodermmaterials sind ganz verschieden von denen des Ektoderms und des Mesoderms. Das Material des Urdarmbodens erweitert sich senkrecht zur Mediane. Aber an den seitlichen Rändern des präsumptiven Entoderms löst sich das Entoderm von dem Mesoderm an der Grenze beider Keimblätter und führt selbstständige Bewegungen aus, d.h. der Randteil des präsumptiven Entoderms breitet sich dünn in seitlicher und kranio-lateraler Richtung aus und bildet das Dach und die Seitenwand des Urdarms. Die vordere Wand des Kopfdarms, mit Ausnahme des mediane Teils, wird daher zum grössten Teil aus dem Material des seitlichen Randes des präsumptiven Entoderms gebildet, und hier ist die Ausbreitung des Entodermmaterials vor allem bedeutend.

Die prächordale Platte, der mediane Teil des Kopfdarms, wurde in diesem Versuch nicht markiert. Aber es ist zu vermuten, dass sie aus dem medianen Material zwischen der Chordaanlage und dem vorderen Ende der Anlage des Urdarmbodens bildet wird. Und hier lösen sich beide Keimblätter nicht voneinander. Denn die prächordale Platte liegt an dem Verbindungspunkt des Mesoderms und des Entoderms.

Experiment Nr. VI.

L. 722. Sechs abwechselnd rote und blaue Marken wurden einem *Lichenatus*-Keim im jüngen Gastrulastadium sechseckig auf der dorsalen Urmundlippe angelegt. Die untere blaue Marke bezeichnete die dorsale Urmundlippe dieses Stadiums (Abb. 45).

23 Stunden nach der Markierung waren die unteren drei Marken, eine blaue und zwei rote, ins Innere eingerollt (Abb. 46). Nach 13 Stunden hatte der Keim einen kleinen Dotterpfropf. Die oberen drei Marken, eine rote und zwei blaue, drängten sich radial um die Blastoporen zusammen

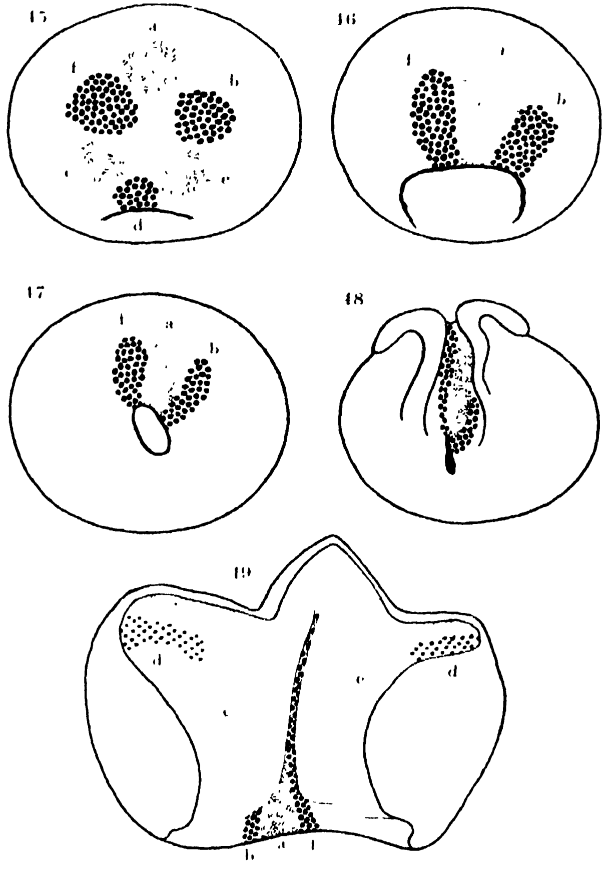

Abb. 45–49. *L. 722.* Markierung im Bereich der dorsalen Urmundlippe. 45: – Anfangsstadium. 46: Nach 23 Stunden. 47: —Nach 13 Stunden. 48: – Nach 72 Stunden. 49: —Eröffnung des Urdarms in Rückenlage durch ventralen Medianschnitt.

(Abb. 47). Nach 72 Stunden war der Keim im Neurulastadium. Die oberen drei Marken, die sich im vorigen Stadium am Blastoporenrand befanden, lagerten sich im hinteren Bereich der Medullarplatte (Abb. 48).

Der Keim wurde in diesem Stadium in Rückenlage durch ventralen Medianschnitt des Urdarms eröffnet. Das vordere Ende des Keims wurde nach seitlichen Einschnitten aufgeklappt. Striche von drei Marken, von einer roten zentralen und zwei blauen seitlichen, wurden an der dorsalen Innenwand des Blastoporus gefunden (Abb. 49). Sie gingen in die Oberfläche der im hinteren Bereich der Medullarplatte gefundenen drei Marken über und tauchten anderseits unter das Entoderm des Urdarmdachs.

Aber weil die Chordaanlage im Neurulastadium noch nicht mit Entoderm beklebt war, sah man die linke blaue Marke, die in diesem Fall auf die Chorda zu liegen kam, zwischen der Lücke beider Entodermnähte hindurch. Die unterste blaue Marke des Gastrulakeims fand sich auf der vorderen Mediane des Urdarmbodens. Sie war in diesem Fall durch den ventralen Medianschnitt mit der Dottermasse beiderseits geteilt. Und kranio-dorsal von ihr wurden zwei rote, bedeutend erweiterte Marken beobachtet. Es sind die roten Marken, die anfangs beiderseits der untersten blauen Marken angelegt worden waren. Sie näherten sich durch seitliche Erweiterung des Entodermmaterials den dorsalen Entodermnähten, aber gelangten nicht bis zu ihnen.

Der vordere Teil der Chordaanlage, die prächordale Platte, blieb ungefärbt. Ihre Lage ist vermutlich in dem zentralen ungefärbten Bereich der oben erwähnten sechs Marken.

Aus diesem Versuch wurde das Lageverhältniss der Chordaanlage und des vorderen Urdarmbodens festgestellt. Auf der dorsalen Mediane liegen die Anlage der Chorda und der prächordalen Platte in einer Reihe. Die Chordaanlage ist in der Höhe des Äquators. Der obere kleine Bereich der Urmundlippe begrenzt den vorderen Teil des Urdarmbodens, und zwischen ihnen lagert sich die Anlage der prächordalen Platte. Daher ist die prächordale Platte das Verbindungsstück zwischen dem Mesoderm und dem Entoderm. An diesem Punkt stehen die beiden Keimblätter, die sich bei den Gestaltungsbewegungen nach verschiedenen Richtungen ausdehnen, in Verbindung.

Experiment Nr. VII

L. 701. Eine Skala von fünf abwechselnd roten und blauen Marken wurden an einem *Lichenatus*-Keim im jungen Gastrulastadium von der dorsalen Urmundlippe bis zur Nähe der oberen Pol auf der dorsalen Mediane

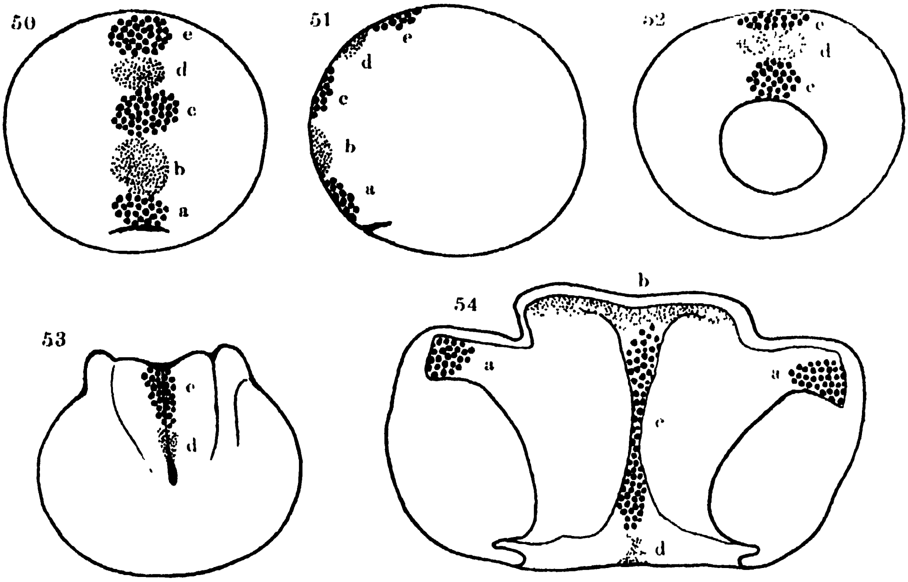

Abb 50–54. *L. 701.* Dorsal-meridionale Markierung einer jungen Gastrula. 50 und 51:—Anfangsstadium. 51:—Profilansicht von rechts. 52: Nach 48 Stunden. 53:—Nach 78 Stunden. 54:—Eröffnung des Urdarms in Rückenlage durch ventralen Medianschnitt.

angebracht (Abb. 50 u. 51). Etwa 48 Stunden nach der Markierung war der Keim im Dotterpfropfstadium. Die untere blaue und rote Marke waren ins Innere eingerollt (Abb. 52). Nach 78 Stunden begann der Keim seine Neurulation. Die oberste blaue Marke verlängerte und lagerte sich in der Medullarplatte. Ein Teil der oberen roten Marke fand sich am Hinterende der Medullarplatte (Abb. 53). In diesem Stadium wurde der Keim in Rückenlage durch ventralen Medianschnitt des Urdarms eröffnet und das vordere Ende des Keims nach seitlichen Einschnitten dorsal aufgeklappt (Abb. 54). Zwischen den beiden dorsalen Entodermnähten fand sich die blau gefärbte Chordaanlage. Aber das hintere Ende der Chordaanlage wird aus dem Material der roten Marke, die in die oberflächliche rote Marke der Medullarplatte übergeht, gebildet. Die prächordale Platte selbst wird von der unteren roten Marke eingenommen. Diese rote Marke zeigte auch merkliche seitliche Ausdehnung. Die anfangs auf der Urmundlippe angelegte blaue Marke fand sich am vorderen Urdarmboden. Sie zeigte auch merkliche seitliche Verbreitung.

Die Ergebnisse dieses Versuchs sind folgende: die prächordale Platte

geht oben in die Chordaanlage selbst und unten in den Urdarmboden über. Sie dehnt sich seitwärts nahe dem Urdarmboden und kranio-kaudal nahe der Chordaanlage aus. Der Übergangspunkt zum Entoderm ist nicht bestimmbar, denn hier geht das Mesoderm allmählich in das Entoderm über, und hier bilden wenigstens an der dorsalen Mediane das Entoderm und das Mesoderm bis zum Neurulastadium niemals eine Spalte. Daher sind die dorsalen Entodermnähte, die vermutlich die letzte Spur der oberflächlichen Spalten beider Keimblätter darstellen, hier nicht mehr sichtbar, hingegen trennen sich am seitlichen Teil des Keims beide Keimblätter während der Gastrulation.

IV. ERGEBNISSE UND BESPRECHUNG.

Aus obigen Versuchen wurde der Anlageplan der jungen Gastrula festgestellt. Er ist bis zum Spätneurulastadium gültig. Für weiter gehende Entwicklungsstadien müsste dieser Plan noch in einigen Punkten ergänzt werden, denn die Versuche wurden diesmal am Ende des Neurulastadiums eingestellt.

Die Einstülpungsgrenze wurde aus Versuchen Nr. I, II, III, VI und VII festgestellt. Sie liegt in der oberen Halbkugel der jungen Gastrula, etwa 15° oberhalb des Äquators an der dorsalen Seite und 10° an der lateralen und ventralen Seite. Aus dem oberhalb dieser Grenze liegenden Keimbezirk werden die Medullarplatte und die übrigen Teile des Ektoderms gebildet. Die präsumptive Lage der Medullarplatte wurde aus den Versuchen Nr. I, II und III festgestellt. Versuch Nr. II zeigt, dass der animale Pol des jungen Gastrulakeims dem vorderen Ende der Medullarwürste entspricht. Und aus Versuch III wissen wir, dass die lateralen Meridiane die laterale Grenze der Medullarwürste berühren. Ferner wurden in Versuchen Nr. I und Nr. II die dorsalen vier Marken immer im Bereich der Medullarplatte gefunden. Daher liegt die präsumptive Anlage der Medullarplatte auf der ganzen dorsalen Seite im Bereich des präsumptiven Ektoderms. Aber sie gehen niemals ventral in die lateralen Meridiane über.

Die Grenze zwischen dem Mesoderm und dem Entoderm wurde durch die Versuche Nr. II, III und IV festgestellt. Sie liegt etwa 20° unter dem Äquator auf der lateralen und ventralen Seite. Auf der dorsalen Seite trennen sich beide Keimblätter nicht voneinander. Sie stehen durch die prächordale Platte in Verbindung miteinander. Die präsumptiven lateralen und hinteren Ränder des Entoderms spalten sich von dem unteren Rand des Mesoderms ab. Das Material des seitlichen Entodermbezirks bildet das Dach und die Seitenwand des Urdarms. Die Spaltlinie des Entoderms

und des Mesoderms entspricht den am Urdarmdach befindlichen Entodermnähten des Neurulakeims. Daher enden diese Entodermnähte in der Nähe der prächordalen Platte.

An der Randzone der jungen Gastrula liegt das präsumptive Mesoderm, und zwar zwischen den oben erwähnten präsumptiven Grenzen gegen das Ektoderm und das Entoderm. An der dorsalen Seite des Mesodermbezirks liegt die präsumptive Chorda. Aus den Versuchen Nr. VI und VII wurde festgestellt, dass auf der dorsalen Mediane die präsumptive Chorda und prächordale Platte in einer Reihe liegen, d. h. in der Höhe des Äquators die Chorda und darunter, zwischen der Chorda und dem oberen Bereich der die vordere Wand des Kopfdarms bildenden Urmundlippe, die prächordale Platte. Die Einteilung dieser Keimbezirke ist schwer, jedoch ist aus den Versuchen Nr. II, VI und VII als annähernd erwiesen anzusehen,

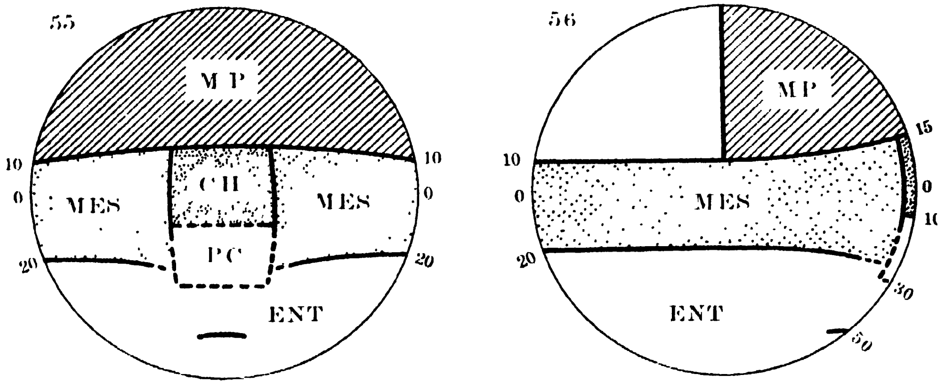

Abb. 55 und 56. Anlageplan der frühen Gastrula von *Hynobius*. Präsumptive Keimbezirke nach der Ergebnisse der Markierungsversuche. 55 von dorsal, 56 von links gesehen. CH: —Chorda. ENT: —Entoderm. MES: - -Mesoderm. MP: - - Medullarplatte. PC: —prächordale Platte. 0°: - Äquator. 50°: – präsumptive Invaginationsgrube.

dass die Chorda im Bereich des dorsalen Mesoderms bis zu 10° unter dem Äquator liegt und die prächordale Platte von 10° ab bis zu 30° unter dem Äquator. Die Breite dieser Anlagen ist gleich und entspricht vermutlich der einen Farbmarke. Sie beträgt etwa 30°.

Die Schemata der Anordnung der präsumptiven Organanlagen des *Hynobius*-Keims zu Beginn der Gastrulation zeigen Abb. 55 und 56. Das gleicht am meisten dem VOGT'schen Schema beim Urodelenkeim. Aber in einigen Punkten weicht es von dem des europäischen Urodelenkeims ab. Erstens ist das Gebiet des Ektoderms, insbesondere auf der ventralen

Seite, beim *Hynobius* kleiner als bei diesem. Die untere Grenze des Ektoderms liegt beim *Hynobius*-Keim immer in der oberen Halbkugel der jungen Gastrula, dagegen nach dem VOGT'schen Schema in der unteren Halbkugel der beginnenden Gastrula. Zweitens ist das Gebiet der präsumptiven Medullarplattenanlage bei jenem auch kleiner als bei diesem. Nähmlich beim *Hynobius* stimmt die Grenze zwischen der präsumptiven Medullatplatte und dem präs. Hautektoderm mit den seitlichen Meridianen überein, beim europäischen Urodelen hingegen dehnt sie sich ventralwärts über die seitlichen Meridianen hinaus. Und drittens ist die Breite der präs. Chordaanlage beim *Hynobius*-Keim auch klein. Im VOGT'schen Schema reicht der obere Teil der Chordaanlage beiderseits fast bis an die seitlichen Meridiane. Aber nach meine Beobachtungen bedeckt die Chorda des Neurulakeims immer nur eine Marke der ganzen Länge nach. Die Breite einer Marke misst etwa 30°. Der von VOGT erwähnte grossen Unterschied in der Breite beim oberen und unteren Teil der Chordaanlage habe ich nicht beobachtet, wenigstens nicht bis zum Ende der Neurulation.

Der Bereich des präs. Entoderms ist bei *Hynobius* gross. Er ähnelt dem Bereich der frühen Blastula von *Triton* und *Pleurodeles* (VOGT '29 Abb. 1 c). Das ist wohl auf die Menge des Dotters zurückzuführen.

Die Bewegungsrichtung der Keimblätter ist ganz gesetzmässig. Das Ektoderm dehnt sich bei der Gastrulation anfangs in meridionaler Richtung aus. Eine horizontal angelegte Serie von Farbmarken in der oberen Halbkugel liegt späterhin meridional nebeneinander um die vertikale Achse der Anfangsgastrula. Und am Ende der Gastrulation werden diese Markenstreifen an der dorsalen Seite dichter als an der ventralen. Mit anderen Worten, das Material des Ektoderm sammelt sich an der dorsalen Mediane, und deswegen werden die ventralen Streifen zerstreut. Kurz, die Gestaltungsbewegung des Ektoderms ist bis zum Ende der Neurulation eine zusammengesetzte Bewegung in meridionaler und latitudinaler Richtung. Am Anfang der Gastrulation ist die Bewegung des Ektoderms ausschliesslich meridionale Streckung. Erst am Ende der Gastrulation wird die Kondensation des Materials an der dorsalen Mediane sehr bemerkenswert. Das ist die dorsale Konvergenz von VOGT.

Die Lageverhältnisse der präs. Medullarplatte bis zur voll ausgebildeten sind, wie folgt: Das vordere Ende der Medullarplatte berührt den oberen Pol der jungen Gastrula, das hintere Ende die untere Grenze der präs. Medullaranlage nach dem darunter liegenden Mesoderm hin. Die seitlichen Medullarwürste berühren die seitlichen Meridiane. Die präsumptive Lage der Rückenrinne der Medullarplatte entspricht der dorsalen Mediane der

jungen Gastrula. Eine Verwachsung der symmetrischen halben Teile der präs. Medullarplatte an der dorsalen Mediane liegt nicht vor. Daher ist Gestaltungsbewegung der Medullarplatte von *Hynobius* nicht Konkrescenz, sondern Konvergenz.

Hier möchte ich etwas zu dem von GOERTTLER definierten Wort „Schwenkung" bemerken. Er sagt: „Unter der Bezeichnung ‚Schwenkung', die ich für diese Art der Materialverschiebung der präsumptiven Medullaranlage gebraucht habe, soll verstanden werden: der Ortswechsel eines in sich geschlossenen Verbandes um einen festen Punkt herum, der in ihm selbst liegt (Schwenkung im militärischen Sinn)" (GOERTTLER '25 S. 509). Und er vergleicht einige Schemata miteinander. Von diesen Schemata bin ich aber nicht befriedigt, wenigstens nicht für *Hynobius* und auch nicht für *Rhacophorus*, worüber ich schon im Jahre 1930 berichtet habe.

Wie oben erwähnt, sind die zwei Richtungen der Gestaltungsbewegungen, die meridionale Streckung und die dorsale Konvergenz, gemäss den Entwicklungsstadien in ihrer Stärke verschieden. Die wirkliche Gestaltungsbewegung ist nichts anderes als die Resultante der beiden Bewegungen. Nun wollen wir zunächst die meridionale Streckung erwägen. Dazu müssen wir in erster Linie ihre Richtlinie, oder mit anderen Worten die Achse feststellen. Ich setze die Achse durch den animalen Pol, der bei der Entwicklung nach vorn hin wandert, und das Zentrum der Keimkugel. Beim Urmundschluss streckt sich das Material der oberen Keimhälfte allseitig gleichmässig nach dem imaginären unteren Keimpol hin. Das bedeutet die meridionale Streckung des Materials gegen die Keimachse hin.

Um dies zu erklären, will ich hier einige Schemata geben. An der Oberfläche einer Kugel zeichnen wir eine horizontale Zone, die von 10° bis 45° über dem Äquator liegt. Diese Zone ist durch acht schraffierte

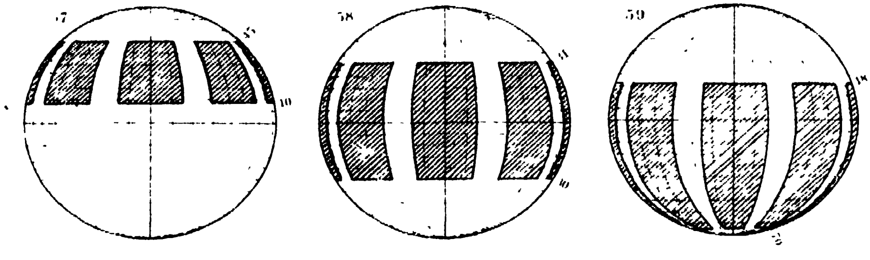

Abb 57–59. Schema. Es zeigt die drei Stadien der meridionalen Streckung des Ektoderms. Weitere Erörterung s. im Text.

Muster um die Kugel herum markiert. Nun vermuten wir, dass dieser ganze 10° über dem Äquator liegende Teil sein Gebiet so gleichmässig ausdehnt, dass die anfangs bei 10° über dem Äquator liegende untere Grenze bis 30° unter dem Äquator sinkt. Dann mag die Vermehrung des Gebiets um etwa 1.8 mal grösser als im Anfang sein *. Die obere Grenze der Zone erreiche 31° über dem Äquator, weil die Polbezirke auch nach der Voraussetzung im Anfang ihr Gebiet im gleichen Verhältnis erweitern. Vermuten wir in gleicher Weise einen Gebietszuwachs bis zu 70° unter dem Äquator, so erreicht die obere Grenze etwa 18° über dem Äquator, und diesmal wird die Vermehrung des Gebiets etwa das 2.3fache betragen.

Mit diesen Schemata wird die meridionale Streckung der schraffierten Muster als Ergebnis der gleichmässigen Vermehrung des Gebiets betrachtet. Beim Vergleich dieser Schemata mit meinen oben erwähnten Beobachtungen (Experiment Nr. 1, Abb. 3 und 5) finden wir grosse Ähnlichkeit unter den Bildern. Vorausgesetzt, dass ich diese Schematisierung nicht falsch beurteilt habe, so ist die Gestaltungsbewegung des präsumptiven Ektoderms der jungen Gastrula zum grössten Teil eine durch im gleichmässigen Verhältnis erfolgende Gebietsvermehrung bedingte meridionale Streckung.

Über die Achsenfrage habe ich schon an einem Anurenkeim *Rhacophorus* meine Ansicht veröffentlicht (MOTOMURA '30). Bei *Rhacophorus* habe ich aus die Messung und Markierung festellt, dass drei Punkte, der oberflächliche Mittelpunkt des Dotterpfropfs und der der Furchungshöhle, oder der obere Pol der jungen Gastrula, und das Zentrum der Keimkugel, eine Achse bilden können, die annähernd mit der vertikalen Achse des Blastulakeims und obenso der Längsachse des ausgebildeten Embryos übereinstimmt, und dass der Urmundrand, dessen Material immer durch Einrollung erneuert wird, sich an den vermutlichen unteren Pol gleichmässig von allen Seiten herandrängt. Gegen meine Ansicht erhob ICHIKAWA ('31) auf Grund seiner Beobachtungen an der gleichen Art folgenden Einwanden. Er sagt: "The downgrowth of the dorsal lip is much quicker than the converging development of all the other parts of the margin of the blastopore and consequently the pore closes at the point midway between the original yolk pole and the spot where the ventral lip first appears". In seiner Veröffentlichung nimmt er den bestimmten Punkt des Dottermaterials als Richtpunkt des Urmundschlusses, ohne die Lage des animalen Pols zu berücksichtigen. Und ferner wurde in seinem Vortrag der genannte Dotterpol durch keine Methode markiert. Er schloss nur aus der Differenz der oberflächlichen Strecke der Äquatormarken auf die

* Der Kreis $x^2+y^2=r^2$ rotiert um x-Achse. Da der Oberflächeninhalt S der Zone des Rotationskörpers, Kugel, gleich dem bestimmten Integral $2\pi\int_a^b y\left[1+\left(\frac{dy}{dx}\right)^2\right]^{\frac{1}{2}}dx$ ist, so folgt:

$$S=2\pi\int_a^b y\left[1+\left(\frac{dy}{dx}\right)^2\right]^{\frac{1}{2}}dx=2\pi\int_a^b r\,dx=2\pi r(b-a)$$

Strecke der Bewegung des Urmunds. Das scheint mir nicht das rechte Mittel zu sein, um die Achsenfrage zu lösen. Schon aus dem Gesichtspunkt der Praxis dürfte es besser sein, wenn der animale Pol, der sich während der Gastrulation und Neurulation an der Oberfläche der Keimkugel befindet, als Richtpunkt der Keimachse festgesetzt wird. Doch müssen zur genauen Beurteilung erst noch die ausführlichen Untersuchungen von M. ICHIKAWA abgewartet werden.

Auch die Neurulation ist geometrisch-schematisch erklärbar. Auf den seitlichen Meridianen setzen wir vom oberen Pol bis zu 10° über dem

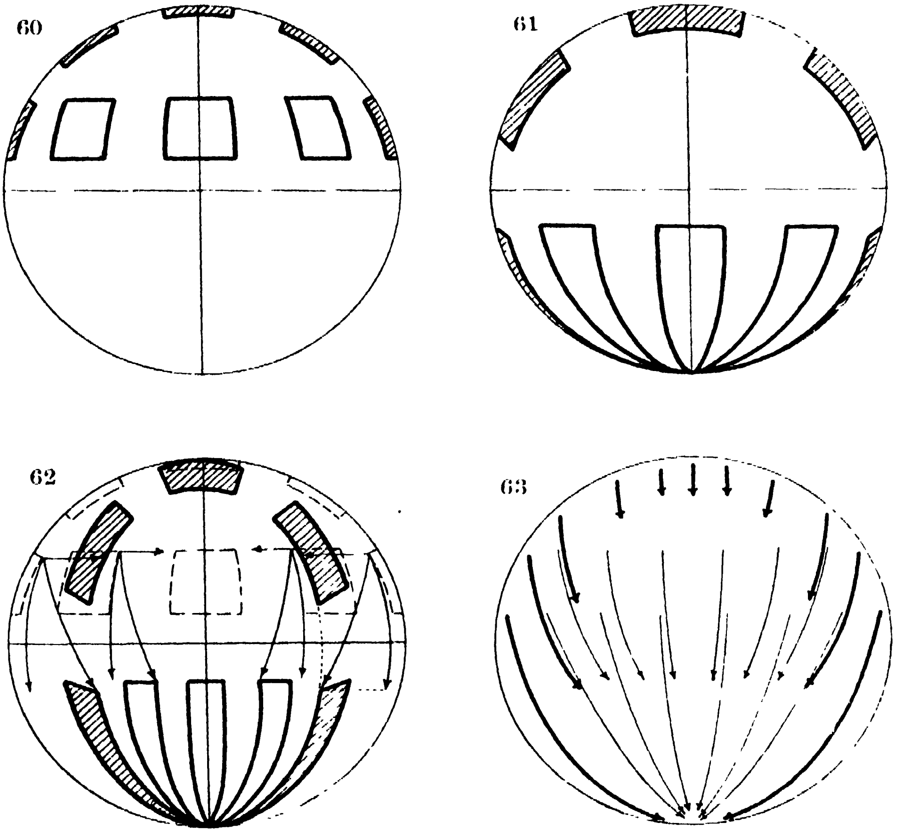

Abb. 60–63. Schema. Es zeigt die Gestaltungsbewegung des Ektoderms bei der Gastrulation und Neurulation. 60:—Anfangsstadien. 61:—Meridonale Streckung des Ektodermbereichs. 62:—Mitwirkung der meridionalen Streckung und der dorsalen Konvergenz. Die Pfeile zeigen die zwei Komponenten, die meridionale und die latitudinale, und ihre Resultanten. 63:—Bewegungsrichtung des Materials der präsumptiven Medullaranlage bei Gastrulation und Neurulation geometrisch-schematisch betrachtet. d. h. die Resultanten der meridionalen und latitudinalen Bewegungen. Die Länge der Pfeile zeigt annähernd die Stärke der Bewegungen.

Äquator eine schmale meridionale Zone, die die präsumptive Medullarwürste bedeutet und in den Schemata durch schraffierte Streifenmuster gekennzeichnet ist, voraus. Wir vermuten auch, dass die unteren Ende der Zone, die sich durch die ganzen Bezirke der Zone nach den oben erwähnten Prinzipien meridional hin erstrecken, bis zum unteren Pol reichen. Nun bekommen wir ein der Neurula ähnliches Bild, wenn der eine von dieser Zone halbierte Teil der Kugel in seiner Breite um eine Hälfte, oder 90°, konvergiert, und die andere Halbkugel in ihrer Latitude so divergiert, dass sie sich um eine Hälfte der anfänglichen Breite, d. h. bis zu 270°, vermehrt. Die Resultanten der zwei Bewegungsrichtungen, die meridionale und die latitudinale, stimmen mit den von GOERTTLER gegebenen Schemata der Bewegungsrichtungen überein (GOERTTLER '25, Abb. 6; und '27, Abb. 2). Auch das auffallende Längenwachstum der Äquatorbezirke der präsumptiven Medullatplatte, das GOERTTLER ('25, Abb. 8) in seinen Schemata zeigte, wird nur durch die meridionale Streckung leicht erklärbar, die durch im gleichen Verhältnis erfolgte Gebietsvermehrung bedingt ist. Bezüglich der Resultate meiner Beobachtungen scheint es mir nahezu richtig zu sein, dass die Materialverschiebung des Ektoderms bei der Gastrulation und Neurulation das Ergebnis zweier selbstständiger Bewegungen, der meridionalen sowie der latitudinalen, ist.

Das Mesoderm rollt sich bei der Gastrulation unter das Ektoderm ein. Am Ende der Neurulation wird der grösste Teil des Ektoderms mit Mesoderm gefüttert, mit Ausnahme des kranio-ventralen Teils. Die Fütterung ist auf der dorsalen Seite stärker als auf der ventralen. Die Bewegungsrichtung des Mesoderms ist anfangs meridional. Die Äquatormarken sind an der umgeschlagenen Seite des Mesoderms als parallele Streifen der Farbmarken zu sehen. Aber die Streifen sind auf der dorsalen Seite dichter als auf der ventralen. Das Mesoderm zeigt nähmlich auch dorsale Konvergenz und ventrale Divergenz.

Die Beziehungen zwischen Mesoderm und Entoderm sind nicht einfach. An der dorsalen Mediane, wo die präsumptive Chorda liegt, stehen beide Keimblätter miteinander in Verbindung. Diese Verbindung wurde bisher prächordale Platte genannt. Aber an der lateralen Grenze lösen beide Keimblätter, wenn die Gastrulation fortschreitet, ihre anfängliche Verbindung. Die Spaltlinie zwischen Mesoderm und Entoderms auf der Seite des Mesoderms liegt vermultlich im vorderen Teil des eingerollten Mesoderms; und die auf der Seite des Entoderms ist die dorsalen Entodermnähte der Neurula.

Die präsumptive Lage des Mesoderms und der Chorda ist der Keimachse

entgegengesetzt, d. h. der künftigen Längsachse des Embryos im Frühgastrulastadium entgegen. Das künftige hintere Ende des Mesoderms und der Chorda entspricht der oberen Grenze der Mesodermanlage des jungen Gastrulakeims. Und der vordere Teil lagert sich darunter.

Die Weise der Gastrulationbewegung des Entoderms ist sehr interessant. Das Entoderm rollt sich anfangs von der dorsalen Urmundlippe, die etwa 50' unter dem Äquator auftritt, ein. Weil nun der dorsale Teil der Invaginationsstelle noch zum Bereich des Entoderms gehört, dringt das präsumptive Entoderm zuerst ins Keiminnere ein. Dieser Teil entspricht dem vorderen Urdarmboden. Die Einrollung schritt beim Entoderm von vorn nach hinten fort. Die lateralen Ränder des präsumptiven Entoderms gehen von der Grenzen nach dem Mesoderm hin aus. Sie wickeln konkav die Urdarmhöhle ein. Beide seitliche Ränder des Entoderms begegnen

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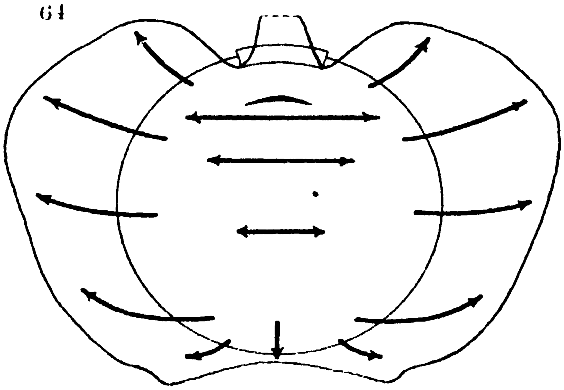

Abb 64. Schema Bewegungsrichtung und ihre Stärke des Entodermbereichs. Der innere Kreis zeigt die präsumptive Entodermbereich in der Oberfäche der jungen Gastrula. Die äussere Kontur zeigt die an der dorsalen Entodermnähte eröffnete Innenfläche des Urdarms.

einander an der dorsalen Mediane des Embryos und bilden die dorsalen Entodermnähte des Neurulastadiums. Diese Entodermnähte verwachsen im Schwanzknospenstadium miteinander. Daher bedeutet die Verwachsung nur beim Entoderm eine Gestaltungsbewegung, beim Ektoderm und Mesoderm hingegen nicht. Aber die Verwachsung der Entodermnähte ist nicht mit der Konkrescenz in den alten Theorien zu verwechseln, denn nach der Theorie der Konkrescenz bedeutet sie eine Verwachsung der zwei anfangs getrennt gefundenen symmetrischen Hälften

Die Ausdehnung und Zusammenziehung in meridionaler Richtung sind

beim Entoderm nicht bedeutend. Aber die Ausdehnung senkrecht zur Mediane ist sehr beträchtlich, besonders im vorderen Teil des Urdarmbodens. Im kranio-lateralen Teil des präsumptiven Entoderms breitet sich das Material sehr stark antero-lateral aus. Im hinteren Teil ist die Richtung meist lateral. Die dünne, dorsale und laterale Urdarmwand wird meist aus den lateralen Bezirken des präsumptiven Entoderms gebildet. Kurz, der wesentliche Punkt der entodermalen Gestaltungsbewegungen ist, dass sich das Entoderm seitlich oder senkrecht zur Mediane ausbreitet und die Urdarmhöhle so einwickelt, dass die beiden seitlichen Ränder des präsumptivem Entoderms an der dorsalen Mediane der Neurula einander begegnen.

Vogt ('29) gibt für Mesoderm und Entoderm bei geschlossener Gastrula zwei Verbindungsstelle an. Er stellte sie durch Schnittbilder fest. Die eine Stelle ist die ventrale Urmundlippe, die zweite die prächordale Platte. Aber, so weit ich nach der Anatomie des Spätneurulakeims urteilen kann, ist der Übergang beider Keimblätter an der ventralen Urmundlippe nicht zu beobachten. Die Einrollung des Mesoderms an der ventralen Urmundlippe war schon eingetreten. Natürlich ist die Frage, wann die beiden Keimblätter an der ventralen Mediane ihre anfängliche Beziehung zueinander lösen. Aber ich kann diese Stelle keine besondere Bedeutung zu erkennen, ausser der, dass die von der kranio-lateralen Entodermgrenzen begonnene Abtrennung des Mesoderms, ganz wie beim Auftreten der Rusconi'schen Spalte, an dieser Stelle zu spät eintritt.

Vorliegende Arbeit wurde im Botanischen Laboratorium der Japanisch-Kaiserlichen Tôhoku Universität auf dem Hakkoda-Gebirge durchgeführt.

V. ZUSAMMENFASSUNG.

1. Der Anlageplan der jungen Gastrula von *Hynobius* wurde festgestellt. Die Einstülpungsgrenze liegt in der oberen Halbkugel der jungen Gastrula, etwa 15° oberhalb des Äquators an der dorsalen Seite und 10° an der lateralen und ventralen Seite. Der animale Pol entspricht dem vorderen Ende der Medullarwürste. Und die lateralen Meridiane berühren die laterale Grenze der Medullarwürste. Daher liegt die präsumptive Lage der Medullarplatte auf der ganzen dorsalen Seite im Bereich des präsumptiven Ektoderms. Aber sie geht niemals ventral in die lateralen Mediane über. Die Grenze zwischen dem Mesoderm und dem Entoderm liegt etwa 20° unter dem Äquator auf der lateralen und ventralen Seite. Auf der dorsalen Seite trennen sich beide Keimblätter nich voneinander. Sie stehen durch die prächordale Platte in Verbindung miteinander. An der Randzone

der jungen Gastrula liegt die präsumptive Lage des Mesoderms, zwischen den oben erwähnten präsumptiven Grenzen gegen das Ektoderm und das Entoderm. An der dorsalen Seite des Mesodermbezirks liegt die präsumptiven Lage der Chorda. Die Chorda liegt im Bereich des dorsalen Mesoderms bis zu 10° unter dem Äquator. Die Breite dieser Anlage beträgt etwa 30°. Die prächordale Platte liegt von 10° ab bis zu 30° unter der Chordaanlage.

2. Die Lagenverhältnisse der präsumptiven Medullarplatte bis zu voll ausgebildeten sind, wie folgt: Das vordere Ende der Medullarplatte berührt den oberen Pol der jungen Gastrula, das hintere Ende die untere Grenze der präsumptiven Medullaranlage nach dem darunter liegenden Mesoderm hin. Die seitliche Medullarwürste berühren die seitlichen Meridiane. Die präsumptive Lage der Rückenrinne der Medullarplatte entspricht der dorsalen Mediane der jungen Gastrula. Eine Verwachsung der symmetrischen halben Teile der präsumptiven Medullarplatte an der dorsalen Mediane liegt nicht vor. Daher ist die Gestaltungsbewegung der Medullarplatte von ***Hynobius*** nicht Konkrescenz, sondern Konvergenz.

3. Die Gestaltungsbewegung des Ektoderms ist zum Ende der Neurulation eine zusammengesetzte Bewegung in meridionaler und latitudinaler Richtung. Am Anfang der Gastrulation ist die Bewegung des Ektoderms ausschliesslich meridionale Streckung. Erst am Ende der Gastrulation wird die Kondensation des Materials an der dorsalen Mediane sehr bemerkenswert: d. h. die dorsale Konvergenz.

4. Das Mesoderm rollt sich bei der Gastrulation unter das Ektoderm ein. Das zeigt auch dorsale Konvergenz und ventrale Divergenz.

5. Die vermutliche Lage des Mesoderms und der Chorda ist der Keimachse entgegengesetzt, d. h. der künftigen Längsachse des Embryos im Frühgastrulastadiums entgegen. Das künftige hintere Ende des Mesoderms und der Chorda entspricht der oberen Grenze der Mesodermanlage des jungen Gastrulakeims. Und der vordere Teil lagert sich darunter.

6. Das Entoderm rollt sich anfangs von der dorsalen Urmundlippe, die etwa 50° unter dem Äquator auftritt, ein. Dieser Teil entspricht dem vorderen Urdarmboden. Die Einrollung schritt beim Entoderm von vorn nach hinten fort. Die lateralen Ränder des präsumptiven Entoderms gehen von der Grenzen nach dem Mesoderm hin aus. Sie wickeln konkav die Urdarmhöhle ein. Beide seitliche Ränder des Entoderms begegnen einander an der dorsalen Mediane des Embryos und bilden die dorsalen Entodermnähte des Neurulastadiums. Diese Entodermnähte verwachsen im Schwanzknospenstadium miteinander.

7. Das Entoderm ausbreitet sich seitlich oder senkrecht zur Mediane und so einwickelt die Urdarmhöhle, dass die beiden seitlichen Ränder des mutmasslichen Entoderms an der dorsalen Mediane der Neurula einänder begegnen.

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THE NERVOUS SYSTEM OF AN ABNORMAL TURTLE EMBRYO, WITH SOME CONSIDERATION OF ITS BEHAVIOR

BY

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EIGHT TEXT-FIGURES

(Received June 16, 1932)

While experimenting on the "Early Behavior of Embryos of the Turtle (*Terrapene carolina* (L))" (TUGE, '31), in the EFFINGHAM B. MORRIS Biological Farm of the Wistar Institute of Anatomy and Biology in Philadelphia, the writer has obtained an embryo ('31 D 1) which was considerably retarded in its growth, especially in the head region.

During the course of observation upon the embryo under the binocular microscope, owing to the small size of the body, *a spontaneous movement of the whole body* was distinctly recognized. Since such spontaneous movement is so unusual for the embryo of this small size, the animal was immediately observed for next five minutes under the binocular microscope, but failed to detect occurrence of perceptible spontaneous movement again.

It would be interesting to undertake an anatomical study upon this abnormal animal, with the hope that such study might throw some light upon our present knowledge of the early spontaneous movement of embryos.

PRENATAL HISTORY OF THE EMBRYO

The egg, which was laid July 8, 1931, was planted in the same manner as the other eggs were. For the experiment upon the embryonic behavior, the egg was opened August 7, 1931 at the calculated age of 30 days. The egg shell was normal in size and color.

The embryo was placed in normal salt solution as usual. The embryo was approximately 3.5 mm. in length. Since the embryos at this stage show no distinct indication of the carapace, it is, in fact, difficult to determine the body length. So the measurement from the top of the midbrain to the base of the tail was made.

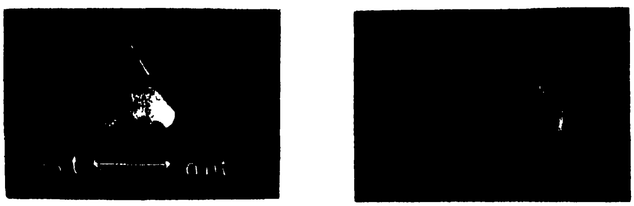
[1)] I am very much indebted to Dr. G. E. COGHILL, for use of his laboratory in the Biological Farm and for supply of the materials.

The heart beats were slightly slower than that observed in the normal embryos, being 52 beats per minute.

A few minutes after the embryo was placed in the solution, it performed a spontaneous movement of entire body (as also with the majority of the normal embryos of this age), which corresponds to that of the total behavior pattern of COGHILL ('29). No excitation was evoked, with this embryo, either by light tactile stimulation with a hair, or by slightly strong stimulation with a bristle, upon various regions of the body.

The embryo was kept in the salt solution for about three hours, and, before death it was fixed in BOUIN's solution, without perceptible contraction.

The embryo, accordingly, may correspond, with some reservation, to the non-motile stage in *Amblystoma* designated by COGHILL ('29).



a b

Fig. 1. Photographs of both the abnormal (*a*) and normal (*b*) turtle embryos (*Terrapene carolina* (L)). Both the embryos were enlarged approximately 2,5 times. The lines adjacent to the embryos indicate the direction of section in figures

In figure 1, *a* and *b*, the normal (*b*) and the retarded (*a*) embryos are shown. The normal embryo ('31 D 2) is of the same age as the abnormal embryo ('31 D 1). Both the embryos had been laid by the same female turtle and buried in the same place. However, no explanation for the retardation could be found in the present case.

METHODS

The abnormal embryo ('31 D 1) was stained *in toto* alum-cochineal and cut 10 μ in thickness. The sections were counterstained with bleu de LYON. The brain and spinal cord were reconstructed at the magnification of 80 times by the blotting paper method of SUSANNA P. GAGE[1], with some slight modification for the present purpose.

In Japan, the thickest sheets of blotting paper obtainable in the market are about 0.4 mm. and so, the two sheets of paper were pasted together

[1] See details in Anat Rec., vol. VII, No. 3, p. 166.

in oder to obtain thickness of approximately 0.8 mm. For cutting accurately along the outline, a paper with penciled drawings was dipped into hot paraffine.

The normal embryo ('31 D 2) was also prepared, as a control, with the same staining method as in the abnormal embryo.

ABBREVIATIONS FOR ALL FIGURES

c. c., canalis centralis
d. aor., dorsal aorta
ear., ear-primordium
gl VII., ganglion of the VII cranial nerve
gl. VIII., ganglion of the VIII cranial nerve
gl. X., ganglion of the X cranial nerve
hem., lateral cerebral hemisphere
isth., isthmic region
lat. v., lateral ventricle of the forebrain
m., neuroblastic hyperplasia in the medulla oblongata
med., medulla oblongata
n., an aberrant nerve
not., notochord
r. III., III cranial nerve
r. VII., VII cranial nerve
r. VII m., motor root of the VII cranial nerve
r. VII s., sensory root of the VII cranial nerve
r. VII m.+s., motor and sensory roots of the VII cranial nerve
r. IX., IX cranial nerve
r. X., X cranial nerve
r. X.+r. IX., combined root of the X and IX cranial nerves
r. X.+r. XI., combined root of the X and XI cranial nerves
sp., spinal cord
tect., tectum opticum
thal., thalamus
v. III., third ventricle
v. IV., fourth ventricle
vis ar., visceral arch

MORPHOLOGICAL OBSERVATIONS

The embryo is characterized by possessing the diminutive anterior part (fig. 1 *a*). It lacks both eyes, and, the mouth opening is not yet established. At the ventro-caudal surface of the head is seen a bud-like prominence, which is formed by aggregation of some visceral arches. These arches are irregular in appearance (figs. 5 and 6, *vis. ar.*).

The trunk region is more developed than the head region. The fore limb buds are not perceptible, but the hind limb buds are well visible. The heart region is swollen, occupying a large portion of the body. The carapace is not yet ridged. The tail is not distinct.

The forebrain is unique, because both the lateral cerebral hemispheres (*hem.*) are narrowly elongated laterad and very small (figs. 2 *a*, 3 and 4). The shape of the ventricle (*lat. v.*) is, therefore, so irregular that it is difficult to identify it as real lateral forebrain ventricle. The terminal plate is not clearly indicated. The olfactory pits on both sides can dificutly be identified, as they are deformed.

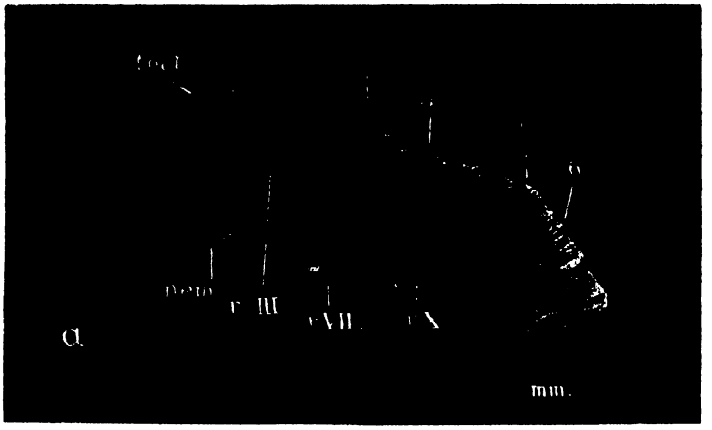


Fig. 2. Photographs of reconstruction model of the brain and spinal cord of the abnormal embryo. *a.*, showing the external view.
b., showing the internal view.
The numerical letters represent the positions of the sections illustrated in figures 3, 4, 5, and 6, respectively. Reduced one-half

The thalamic region (*thal.*) is much larger as compared with the cerebral hemisphere (fig. 4). No distinction between the thalamic and hypothalamic regions can be made. A glandular outgrowth regarded as pineal body exists at the ordinary position, but it is very small.

Neither the optic stalks nor the optic vesicles are found. The lens formation, moreover, is not detected.

The midbrain is small and undeveloped, owing to the lack of visual

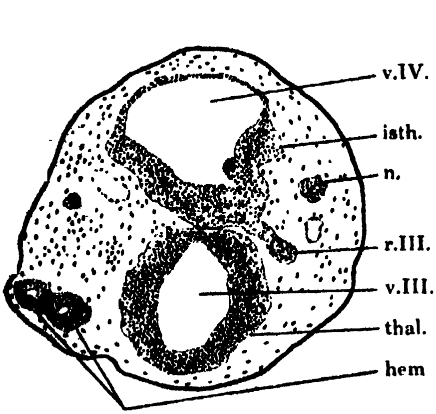


Fig. 3. Transverse section through a level of the oculomotor nerve (*r.III.*). Attention should be directed to the peculiarly elongated lateral cerebral hemisphere (*hem.*). × approximately 66.

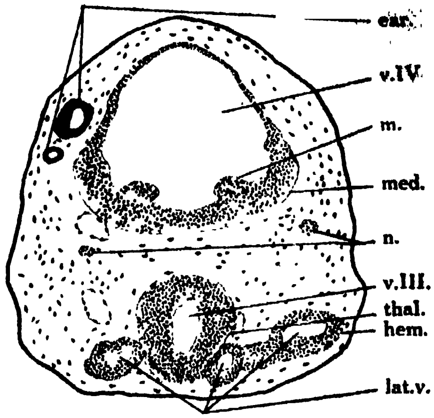


Fig. 4. Transverse section through a level of the middle portion of the medulla oblongata. Attention should be directed to the elongated lateral cerebral hemisphere (*hem.*) as well as in figure 3, and also to the neuroblastic hyperplasia (*m.*) in the medulla oblongata. × approximately 66.

organs (fig. 2, *tect.*).

The medulla oblongata is relatively normally developed in shape, though smaller in size (figs. 2 *b*, 4 and 5, *med.*).

The spinal cord in the trunk regions is highly developed (figs. 2 *b* and 7, *sp.*). The spinal cord at the tail region, on the other hand, is distorted because of the imperfect formation of the tail.

Concerning the cranial nerves and their ganglia, many peculiarities are shown.

The olfactory and optic nerves are lacking. The

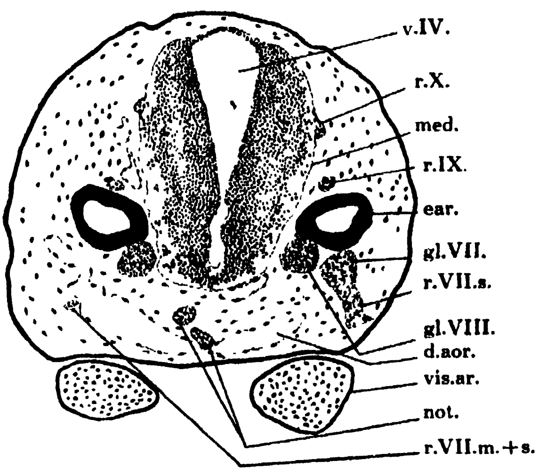


Fig. 5. Transverse section through the caudal region of the medulla oblongata. Attention should be directed to the nervous components of VII, which innervate the several visceral regions and skin (*r.VII.s.* and *r.VII.m.+s.*). Note also a condition of proliferation of cells in the medulla oblongata. × approximately 66.

oculomotor nerves (*r. III.*) on both sides emerge, as thick bundles, in the normal position, though not strictly symmetrical (figs. 2 *a* and 3). The oculomotor nerves disappear after extending a short distance, due likely to the absence of the corresponding eye muscles.

The trochlear nerves do not exist, in association probably with the absence of the superior oblique muscles.

At the level of figure 4, the large trigeminal nerve and its ganglion should have appeared, if the embryo were not abnormal. The trigeminal nerve components, however, do not seem to make any appearance at all. This may be interpreted as due to the fact that the jaw and the receptors distributed in the head region, including the nose and mouth, are very poorly developed. As will be shown later (see, p. 417), the nervous components innervating the muscle and skin, which in my mind correspond to that of trigeminus, come into close relation with the facial components which reach the greatest degree of differentiation. (Figs. 5 and 6, *r. VII. s.*, *r. VII. m.*). Furthermore, a pair of masses consisting of neuroblasts is seen to evaginate towards the fourth ventricle at the level in question (figs. 2 *b* and 4, *m.*). This peculiar evagination (hyperplasia) of neuroblastic masses would be assumed to be the results of the abnormal development of the trigeminal nervous complex, and should have formed such as the Gasserian ganglion.

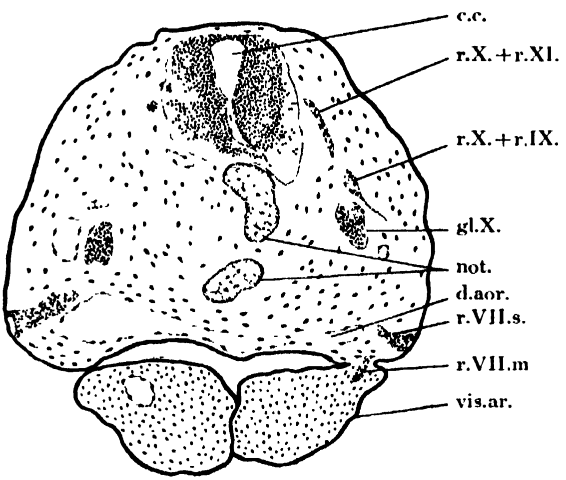


Fig. 6. Transverse section through a level transitional from the medulla oblongata to the spinal cord. Attention should be directed to the nerve roots of X (*r.X.+r.IX.* and *r.X.+r.XI.*) and its ganglion (*gl.X.*) and the peripheral innervations of the nervous components of VII (*r.VII.m.*, *r.VII.s.*). Note a remarkable condition of proliferation of cells at this region. × approximately 66.

The abducens nerves are not found at all, and at the same time the lateral rectus muscles are not formed.

The motor component of the facial nerve is seen to emerge from the portion of the motor VII nucleus. A

great number of nerve components enter extra-cranially the ganglion of the facial nerve (*gl. VII.*) and proceed towards the periphery accompanying those sensory components which form the ganglion (figs. 2 *a* and 5, *r. VII.*). Soon after some components of the nerve branch out of the ganglion, being separated into a few branches. Some of them proceed towards and innervate various visceral regions. They perform possibly both general viscero-motor and special viscero-sensory functions (fig. 5, *r. VII. m.+s.*), and the others advance towards the facial region. The third (*r. VII. m.*) go ventrad to innervate some embryonic visceral arch (*vis. ar.*) which may be the hyoid arch (fig. 6). As mentioned above, a bud-like prominence, which was probably formed by fusion of some visceral arches, is extensively innervated by those branches of the motor VII nerve component. There is, furthermore, found a large nerve of the VII component, which innervate directly the skin of the ventro-caudal part of the head region (figs. 5 and 6, *r. VII. s.*). From the fact mentioned above, it seems reasonable to consider that the peripheral trigeminal innervation was largely compensated by the VII nerve components.

In this connection, a pair of unknown nerves (*n.*) which emerge in front of the level of figure 3 will be considered. Each root of the nerve in question on either side shows asymmetrical arrangement (figs. 3 and 4, *n.*). The nerve root on one side is apparently in connection with one of the branches of the VII nerve at the level of this ganglion. These nerves can not be identified with any missing cranial nerves in the embryo. These may be comparable with an aberrant nerve which has been found in experimented *Amblystoma* by BURR ('30).

In contact with the ganglion of the VII nerve, another ganglion (*gl. VIII.*) appears, which comes anatomically into closer relation to the ear-primordia (*ear.*) than does the ganglion of the VII nerve (fig. 5). This ganglion may be regarded as the ganglion of the VIII nerve. The intra-cranial nervous connection to this ganglion, however, is not traceable.

The IX nerve components (*r. IX.*) will be seen to emerge from the normal position (fig. 5). After coming out of the medulla oblongata, they seem to come into close contact with the ear-primordia, and then fuse with the nerve components of X (fig. 6, *r. X.+r. IX.*, *gl. X.*).

The development of the nerve X is remarkable (fig. 2 *a*, *r. X.*). It is seen to join rostrally with one of the branches of the IX and form the ganglion of the X nerve (fig. 6, *gl. X.*). Many nerve roots emerge from the ganglion and are traceable to the heart region and other visceral regions.

The distinction among the X and XI and also dorsal spinal nerves was not clear from one another (fig. 6, *r. X* + *r. XI.*), and they probably form mixed components at the transitional level from the bulb to the cord (fig. 6). At any rate, the branches of these nerve roots at the region mentioned above, are well developed in appearance, and, therefore, suggest that the viscero-sensory and possibly viscero-motor components were, to a certain extent, functional.

I have mentioned in my paper ('32) that in the adult turtle the anterior roots of the hypoglossal nerve will mostly innervate the tongue muscle, while the posterior ones will mostly innervate the neck muscles (p. 245). The anterior part of the hypoglossal nerve, in this abnormal embryo gives off a single component at the position which corresponds to that of the posterior part of the same nerve in the normal brain, and also, the part, which corresponds to the hypoglossal component for the tongue muscle (or, to the anterior part of the normal brain), may very likely be lacking due to the absence of the tongue muscle. At the same time, an emphasis will be given to the presence of the neck muscles, though they are not well developed. The condition obtained from the embryo would indirectly support the view that in the normal adult turtles the anterior part innervates the tongue muscle and the posterior part innervates the neck muscles.

It was also difficult to make distinction between the posterior part of the hypoglossal nerve and the spinal somatic motor components at the region concerned, as would be seen in the case of certain animals; as, for instance, in some of reptiles and amphibia.

The spinal somatic motor components, however, attain the greatest degree of development as it proceeds caudally, and innervate the muscle segments in the trunk region.

The formation of the muscles of the fore limbs is in less degree of development, whereas the peripheral nerves are seen to proceed towards the limb, though their innervation to the muscles was not distinct.

As already mentioned that the hind limbs are advanced conspicuously in development, the peripheral nerves to the hind limbs are, accordingly, considerably thick and branch out so as to innervate all the parts of the limbs. In the functional center of the hind limb within the spinal cord, a rapid proliferation of the nerve cells is seen. The cord at the region concerned is of enormous size.

The spinal ganglia are well arranged segmentally (fig. 7, *d-k*) and their central connection appears to be well established. The peripheral distribution of the spinal sensory roots is extensive. These anatomical features

are not so different from the condition found in the normal embryo (figs. 7 and 8).

Furthermore, the sections of the cord taken at several levels show advanced proliferation of the cells in both motor and sensory regions (Fig. 7). The complexity of the processes of these cells is also remarkable, particularly those in between sensory and motor regions, as will be described later (see p. 420).

The size of the sectional areas of the cord at several levels does not appear smaller than that of the normal embryo. The reader will be able to compare the approximately corresponding sectional areas of both embryos (figs. 7 and 8).

Finally, observations upon the conduction paths in the medulla oblongata and spinal cord are described without detail.

Both motor and sensory longitudinal tracts, particularly in the motor zone, make their appearance as marked tracts at the periphery of the bulb. These tracts are seen in the rostral part of the isthmic region. The more the sections proceed caudally, the more

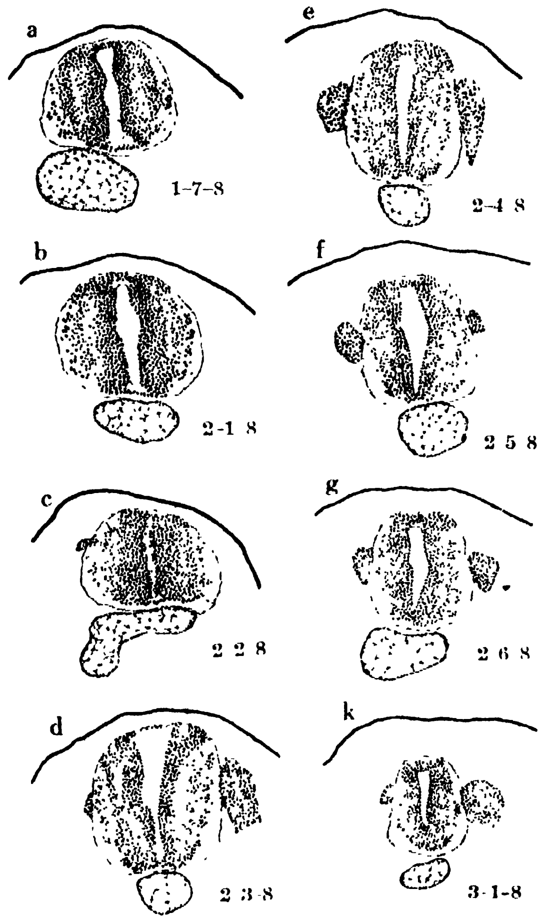


Fig. 7. Transverse sections (*a k*) through various regions of the spinal cord. The sections begin from a level at the most caudal portion shown in the reconstruction model (fig. 2) and end near the base of the tail region. These (*a–k*) are taken from each 18th section. Attention should be directed to the advanced degrees of proliferation of cells in the spinal cord and also to the well-developed spinal ganglia (*d–k*). In contact with the spinal cord the notochord is shown. × approximately 66.

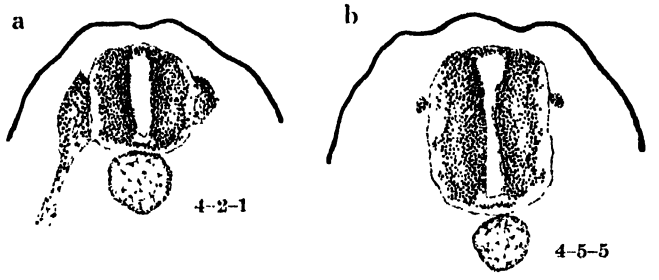


Fig. 8. Transverse sections through the two different regions of the spinal cord of the normal embryo ('31 D 2), showing a comparison of the sectional areas and proliferation of cells with those of the abnormal embryo ('31 D 1) in figure 7. *a* (4-2-1) corresponds approximately to *b* (2-1-8) of figure 7, and *b* (4-5-5) approximately to *a* (1-7-8) of figure 7. × approximately 66.

these tracts become numerous and remarkable. On the contrary, it is evident that in front of the isthmic region there are seen very few conduction paths from or to the lower centers. That is to say, the fore- and midbrain are related to the bulb and cord by very few conduction paths in this embryo.

Near to the midline in the motor zone of the bulb, there are grouped a few longitudinal tracts. These tracts are seen at a short distance beyond the level of figure 4, though these are not so remarkable there. Perhaps these tracts constitute some of the fasciculus longitudinalis medialis, judging from the position and structure shown by the normal embryos at this stage.

The fasciculus longitudinalis medialis is believed to originate not only from some centers in the thalamic, hypothalamic region, and midbrain, but also from many nuclei of the medulla oblongata (TUGE, '32, pp. 253, 254, etc.). In the present abnormal embryo, however, the fasciculus longitudinalis medialis may not be related to those higher centers, as it fails to show its presence at these levels, but mostly to the bulbar nuclei. Therefore, it is further noted that the fasciculus longitudinalis medialis makes a marked appearance as the sections proceed caudally towards the spinal cord. In the spinal cord, it may be said that the fasciculus is almost in the same degree of development as found in the normal embryos at this stage.

Besides these longitudinal conduction paths, many fibers connecting the dorsal (sensory) and the ventral (motor) areas in the bulb and cord are as well-developed as they are in the normal embryos. These fiber system would, to some degree, have attained the ability to function, and, in fact, this condition of development should be required in producing the eariest nervous movement (neuro-muscular).

DISCUSSION

ALSOP ('18–19) experimented upon chick embryos placing them at normal and abnormal temperatures, and found that some of embryos developed abnormally in size, even when incubated at normal temperature. He concluded, therefore, that abnormality in size might be due to heredity (p. 311).

COGHILL ('29) noted that the anterior part of *Amblystoma* is first destroyed and then the posterior part, when the animal was put in certain solution of potassium cyanide (p. 42), similar as I have found in the abnormal turtle embryo.

The present writer ('31) has observed, in the course of the experiment upon a number of the turtle embryos, that the spontaneous movement of the total pattern occurs before the time when the embryos begin to respond to tactile stimulation.

The same was observed by a number of investigators; PATON ('07 and '11) in amphibia and fishes, WINTREBERT ('20) in selachian, MINKOWSKI ('28) in human fetuses, TRACY ('25 and '26) in teleosts, KUO ('32) in chick embryos, and others.

TRACY ('26), for instance, states explicitly that "the first movements of the embryos take place under condition such that the action of outside stimuli is apparently excluded." (p. 258).

Therefore, it seems to me there is no room to doubt that the spontaneous movement of the total pattern can occur prior to the reaction to stimulation from outside the body.

In the normal turtle embryos, only careful observation with the aid of a binocular microscope will enable us to see the regular rhythmic movement identical with the heart beat. The amplitude of the rhythmic movement in the turtle embryos, however, does not appear so pronounced as the others reported for selachians (PATON and WINTREBERT) and for aves (KUO), though conditions when observed by them may not be identical with my own.

At a later stage, there appears abrupt and irregular movement of the whole body. The data obtainable in the turtle embryos seem to suggest that such movement, abrupt and irregular in character, is due to nervous impulses, and it can be hardly considered as direct muscle contraction, because it occurs simultaneously in different muscle segments. This might agree with TRACY's observation (TRACY, '26) in which he showed that "the *first* movement is always irregular"[1] in the teleosts (p. 264).

[1] Insertion in Italics is made by the present writer.

It should be emphasized, therefore, that the earliest movement of embryos begins with the passive rhythmic movement due to the heart beat as mentioned by Kuo ('32, p. 409), and, later, is added the abrupt and irregular movement (neuro-muscular). However, according to developmental pattern of various animals, these orders given above may be more or less modified.

With the abnormal turtle embryo in question, notwithstanding its considerably small size of the body, anatomical observations showed that the medulla oblongata attains almost to the same degree of development as in the normal embryo. As already noted, the cranial nerves of the somatic motor system indicate strikingly poor development in the medulla. We have evidence, however, that the function of these nerves above mentioned may really do nothing with nervous spontaneous movement of the total pattern.

Tracy ('26) found the occurrence of the sufficient spontaneous body movement of the teleosts, in which the eyes and olfactory organs had been destroyed before the first body movement appeared (pp. 268 and 285). This fact seems to indicate that the somatic motor nerves which are related to the movement of the eye-ball as well as the eye itself, are not essential for the early nervous spontaneous movement. Also the lack of the anterior part of the hypoglossal nerve may have no bearing upon eliciting the movement (see, p. 418).

In the present case, the bulbar sensory parts of VII, X and possibly others should be considered as to have played much important rôle, since the visceral sensory innervations of these nerve components are extensively well established towards effecting the early nervous spontaneous movement.

In the spinal cord, the somatic motor nerves as well as the spinal sensory nerves reach to an advanced phase of development as has been already described. The degrees at which cells are proliferating to function in several levels between the cord of the normal embryo and that of the abnormal, is essentially the same.

Nicholas ('30) has made the interesting observation that, in *Amblystoma* embryo operated for the mesencephalic removal, " the early reactions are absolutely independent of fore- or mid-brain connections " (p. 7). The fact secured by Nicholas seems to be practically parallel with the occurrence of the nervous spontaneous movement observed by the present writer. Furthermore, his observation showed that, following the mesencephalic removal, no reduction in cell number within the cord was found. On the contrary, when the medulla is removed, reduction in cell number within

the cord was remarkable (p. 14, and '29, p. 99). He, therefore, concluded that there exists "the dominance of the medulla as the effective agent in controlling by its descending fibers the proliferation of cells within the spinal cord during its morphogenesis." ('30, p. 20). The bearing above mentioned seems to be similar with the fact that the rapid proliferation of cells in the spinal cord is found in the abnormal embryo, independently of the less development of the fore- and midbrain.

Finally, following the results obtained from the abnormal embryo, the present writer agrees with SWENSON ('28) in the rat fetuses, who claims that the size of embryos alone can not be taken "as a standard of the degree of structural development."

SUMMARY

1) The abnormal turtle embryo was about half size of the normal embryos at the same stage (30 days old), and was observed the occurrence of a spontaneous body movement.

2) The fore- and midbrain are considerably small and many structural defects and deformities are pointed out, as follows; undevelopment of the nasal organs, distorted lateral cerebral hemispheres, lack of the whole visual organs, and so forth.

3) The medulla oblongata shows good development, but the cranial nerves of IV, V and VI are lacking. The nervous components of VII and X attain to the greatest degree of development.

4) The size of sectional areas and the degree of cell proliferation in various regions of the spinal cord indicate almost the same phase of development as that in the normal embryo. Also, concerning the spinal nerves and their ganglia, the same may be said.

5) The spontaneous movement of the total pattern occurs prior to the time when the embryos begin to respond to tactile stimulation (or outside stimuli).

6) The occurrence of the spontaneous body movement of the abnormal embryo is suggested to be due to the developmental phase of the medulla oblongata and spinal cord, independently of the fore- and midbrain.

7) The size of the animal does not determine the degree of the structural development in embryonic behavior.

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A NOTE OF THE EARLIER STAGE OF COLONY FORMATION WITH THE CORAL, *POCILLOPORACESPITOSA* DANA.*

By

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During my sojourn in the Palau Islands (October, 1930—March, 1931) for the purpose of collecting corals, some were found growing on the bottom of a ship anchored for a long time. This fact interested me to investigate in what manner the coral planulae settle on the solid substratum.

The settlement of free-swimming planulae was tested on the surfaces of a concrete disk which is 20 cm. in diameter and 2.5 cm. in thickness, which was exposed to rain for about two months previous to use in order to remove the injurious effect to living matters. The disks were hanged either horizontally or vertically in the sea water, utilizing the bamboo raft fixed with anchors as a bouy.

On October 15, 1930, the disks were suspended with wire in various depths; 0 m. (surface), 0.5 m., 1 m., 2 m., 3 m., 5 m., 7 m., and 10 m. In the present paper the results obtained from the disks hanged at the depths of 0 m., 0.5 m., 1 m., 2 m., and 3 m., are reported, for since molluscs, algae, and fine sediment rapidly covered the surface of the disks in deeper water, leaving no room for the attachment of the young coral and hence I was obliged to leave the islands on account of limited time.

The tests were made at the widened portion of a narrow canal between the reef platform surrounding the islands where numerous colonies of various kinds of corals are growing. The depth was about 6 fathoms, and the water temperature measured in the daytime at that time was 29.2°C. in average, varying from 28°C. to 31°C.

OBSERVATION

On December 10, 1930 I discovered the first young of the coral on the disks after the commencement of the experiment. At that time, I

*Contributions from the Marine Biological Station, Asamushi, Aomori-ken. No. 89.

found 10 colonies belonging to one species of the Madreporarian corals. They were found settled on the disks except those placed at the depths of 5 m., 7 m., and 10 m.

The disks were examined on December 31, 1930, January 21, 1931, and February 20, 1931, and the number of colonies were found to have increased at each examination.

TABLE I.

A. Number of the colonies attached to the vertical disks.

Depth	Dec. 10, 1930	Dec. 31, 1930	Jan. 21, 1931	Feb. 20, 1931
0 m.	1	2	2	3
0.5 m.	–	1	2	2
1 m.	1	3	3	3
2 m.	2	5	5	8(1)
3 m.	1	2	3	3
Sum	5	13	15	19

B. Number of the colonies attached to the horizontal disks.

Depth	Dec. 10, 1930	Dec. 31, 1930	Jan. 21, 1931	Feb. 20, 1931
0 m.	1	2	3	3
0.5 m.	2	5	5(1)	5
1 m.	1	1	3	4
2 m.	—	2	1(1)	1
3 m.	1	2	2(2)	4
Sum	5	12	14	17

The number in the parenthesis is that of the detached colonies.

All of the corals belonged to one species, *Pocillopora cespitosa* DANA. This species which is much branched is common in the shallow waters of the Palau Islands, being especially abundant on the reef platform near the station. This is also known distributing in the Hawaiian Islands, Fiji Island, Tahiti, Ceylon, etc. Therefore, it is one of the most common species of the Madreporarian corals growing in the coastal area of the tropical seas.

The adult colonies of the species are much smaller as compared with the other species of the Genus *Pocillopora* found in the Palau Islands.

The fresh color of the soft part is generally greyish brown, though in some the apical parts of the tentacles exhibit a greenish color.

EDMONDSON (1929) in his recent paper has reported on the development of the earlier stages of this kind of the corals obtained from the coral reef of the Hawaiian Islands, and STEPHENSON (1930) also on that of *Pocillopora bulbosa* from the Great Barrier Reef. Although it was stated by these authors that the planulae of the species of the Genus *Pocillopora* were observed, but in the present water, free-swimming ones were not found, in spite of careful examinations of the plankton collected from the regions near the raft, or those scattering from the matured colonies of *Pocillopora cespitosa*. STEPHENSON (1930) also did not detect with certainty any planula belonging to *Pocillopora bulbosa* in the plankton.

When the tide went, I saw a considerable number of *Fungia actiformis* variety, of which more than 50 individuals were found in an acre meter at the most abundant region of the reef platform, emitting actively numerous free-swimming planulae, but I did not find a single planula in the plankton obtained by means of tow-netting when the tide came. It is highly probable that planulae of the corals in nature usually develop in the parent coelenteron till advanced enough for fixation, and then they come out in the sea water to attach as soon as possible to the suitable substrata as STEPHENSON (1930) suggested.

EDMONDSON (1929) found planulae of *Pocillopora cespitosa* in the laboratory during January. STEPHENSON (1930) emphasized that the emission of planulae of *Pocillopora bulbosa* is connected with the phases of the moon. My own present study seems to indicate that planulae of *Pocillopora cespitosa* in the Palau Islands are extruded probably throughout the year, since during the months in which the observations were continued the number of colonies of the coral increased at each examination.

The young corals are able to fix themselves on the both faces of the disks which were placed vertically, though the majority of planulae settled on the upper half of the disk. In the horizontal disks the attachment of all colonies occurred only along the margin owing undoubtedly to the injurious effect to sediment deposited on the disk as many previous investigators have already proved.

Some colonies once settled evidently disappear either by death or by detachment. The number of the colonies detached from the disks was 5% on the vertical disk and 24% on the horizontal disk (Table I) in my experiments. The disappearance of the young coral was certainly caused partly by shells and bryozoa which are the serious persecutors to the

development of the coral as well as by fine sediment which covers the colonies.

The number of the colonies increased more rapidly during December than during January and February, that is to say, the emission of planulae of *Pocillopora cespitosa* was more active in December than in the other months, so far as tested.

The smallest size of the colony which I found on the disk was 1.5 mm. in diameter consisting of three polyps. At the beginning of the development, the colonies spreaded laterally over the surface of the disk. When a colony involves more than 30 polyps, they usually grow upwardly and rise above the basal flattened parts, and then begin to branch. The vertical growth generally starts at the time when the colonies attain about 30 polyps, but I noted some exceptional cases in which a colony showed a vertical growth with only 22 polyps and in other colonies the growth was laterad yet forming the basal plate with as many as 41 polyps. The sizes of the colonies are variable during the earier flattened enlargement stage according to the number of the polyps (Table II).

TABLE II.

Showing the relation between the number of the polyps and the size of the colonies during the basal plate formation.

Number of polyps	Number of examined colonies	Average length of longer diameter	Average length of shorter diameter
3	2	1.5 mm.	1.5 mm.
4	2	2.0 „	2.0 „
5	2	2.3 „	2.2 „
6	3	2.6 „	2.5 „
7	4	2.8 „	2.5 „
8	3	3.0 „	2.8 „
9	2	3.2 „	2.5 „
10	6	3.2 „	2.9 „
11	3	3.5 „	2.8 „
12	3	4.1 „	3.7 „
13	1	3.5 „	3.0 „
14	2	4.0 „	3.8 „
15	2	4.5 „	4.0 „
16	6	4.1 „	3.8 „
17	3	4.5 „	3.8 „
18	1	5.0 „	3.8 „

Number of polyps	Number of examined colonies	Average length of longer diameter	Average length of shorter diameter
19	1	4.0 mm.	4.0 mm.
20	2	4.7 ,,	4.7 ,,
22	2	5.0 ,,	4.5 ,,
23	1	5.5 ,,	5.0 ,,
24	1	5.0 ,,	4.5 ,,
26	2	5.8 ,,	4.8 ,,
27	1	6.0 ,,	5.5 ,,
28	1	6.0 ,,	5.5 ,,
29	2	5.8 ,,	5.5 ,,
30	2	5.3 ,,	5.0 ,,
31	2	5.3 ,,	5.0 ,,
32	1	6.0 ,,	6.0 ,,
33	1	6.0 ,,	5.5 ,,
34	1	5.5 ,,	5.5 ,,
35	1	6.5 ,,	6.0 ,,
38	1	7.0 ,,	6.0 ,,
41	1	7.5 ,,	6.5 ,,

Concerning the increase of the number of the polyps EDMONDSON (1929) stated that the specimens of *Pocillopora cespitosa* which grew most favorably in the laboratory, attained 20 to 25 polyps in the first year. My own observation with the same species in nature showed that the buds are added with very rapid rate; that is the average increase of the polyps being 0.46 per day during the stage of the basal plate formation and 1.45 per day after the beginning of the vertical growth. We further note that the number of the polyps of the specimens which have already commenced the vertical growth, increases about three times as fast as that of the colonies which are still in the lateral extension. Indeed I have even found a specimen which added about 5 buds in two days, though on the contrary there was an example which showed no increase of the polyps in the earlier stage during the observation.

Final examination showed that among all the colonies, one which extended most widely measured 12 mm. in longer diameter and 11 mm. in shorter diameter. One with the greatest number of polyps in the colony was 112. The colony which grew best vertically was 5 mm. high and its raised part consisting of 53 polyps, showing an indication of the commencement of the three-forked development at the tip.

In the present experiment I did not find the colonies formed by fusion

TABLE III.

An example of the rate of increase of polyps per day in the colonies attached to the disk suspended horizontally at the depth of 0.5 m.

No. of colonies	Dec. 10- Dec. 31	Dec. 31—Jan. 21	Jan. 21—Feb. 20
I	0.29		
II	0.38	0.52	2.36
III		0.86	1.73
IV		0.38	0.70
V		0.05	0.23
VI			0.01

The colony No. I was not found when the disk was examined on January 21, 1931. Colony No. II already commenced to grow vertically on December 31, 1930 and No. III on January 21, 1931.

of two or more specimens as STEPHENSON (1930) observed in *Pocillopora bulbosa*, though the fusion of the young of *Pocillopora cespitosa* was occasionally noted as I also noted it sometimes occurring in other corals.

SUMMARY

1. The planulae of a single kind of the coral named *Pocillopora cespitosa* DANA are usually found attached to the floating substrata in the sea of the Palau Islands. The planulae are extruded throughout the year, most actively during December, though free-swimming ones were not found in the plankton collected by netting from the region where numerous adult colonies of this corals are growing.

2. The planulae of the coral do not fix themselves on the surfaces of the disks suspended horizontally in the sea water, due to the fine sediments deposited on the disks. Shells and *Bryozoas* are also the enemies to the young colonies of the coral.

3. The attached planulae grow at first laterally over the surface of the solid substrata, so as to form the basal plate, but when the colonies attain more than 30 polyps, then they usually begin to grow vertically above the basal plate.

4. The sizes of the basal plates vary with the number of the polyps until the beginning of the vertical growth.

5. In the earlier stage of the colony formation the rate of the increase in the number of the polyps per day is about three times as fast during

its vertical growth as in the stage of the basal plate formation.

Taking this opportunity, I wish to extend my sincere appreciation to Prof. S. HATAI for his aid rendered me during this investigation, and also to both late GOSUKE YOKOTA, Governor of the South Sea Islands and to the SAITO Gratitude Foundation for enabling this research.

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GROWTH RATE OF REEF BUILDING CORALS, INHABITING IN THE SOUTH SEA ISLAND.[1]

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INTRODUCTION

Concerning the problem of the coral reef formation, the growth rate of those corals which participate in reef building must be determined and indeed many investigators estimated the growth rate of the corals in different localities of the following oceans.

Pacific Ocean	Murray Island	MAYER	1918
,,	Funafuti	FINCKH.	1904
,,	Samoa	MAYOR	1924
,,	Hawaii	EDMONDSON	1929
Indian Ocean	Cocos	GUPPY	1889
,,	Laccadive	GARDINER	1903
,,	Cocos	WOOD JONES	1910
Atlantic Ocean	Havana	AGASSIZ	1890
,,	Bahama	VAUGHAN	1907
,,	Tortugas	CARY	1904

In the present paper the results of our studies on the growth rate of reef building corals in the South Sea Islands, namely: Yapp and Palau, are presented. For this purpose TAMURA stayed in Yapp Island from October 1929 to March 1930 and observed the growth rate of 12 common species of corals, involving 8 species of branching corals, 2 species of massive corals and 2 species of mush-room corals. HADA stayed in Palau Island from September 1930 to March 1931 and measured the growth rate of 7 species of mush-room corals and one species of the massive form coral.

We wish to express our deep obligation to Prof. S. HATAI and Assistant Prof. S. KOKUBO under whose supervision this work was carried out. Thanks are also due to the governer of the South Sea Islands, and to the SAITO HOONKAI for the defrayment of the greater part of the expenses

[1] Contributions from the marine Biological Station, Asamushi. No. 90.

required in the present study, and we are much indebted to Mr. T. SUGIYAMA, who identified most of the species of the corals employed in the present investigation.

1) GEOGRAPHICAL AND METEOROLOGICAL CONDITIONS

Yapp is a small oceanic island belonging to the group of Calorine Islands and is about 180 sq. km. in area. This island is nucleated with volcanic rocks and is encircled with coral reefs, thus showing an appearance of a kind of fringing reef island. It is situated 138°.8 E. Log. and 9°.30 N. Lat.

The climate of this island is oceanic, showing the air temperature of 25°.1 C. in minimum and 26°.4 C. in maximum in monthly mean through the year. The following table shows the results of the meteorological observations made by the government officers of Yapp in 1927.

The temperature of the sea water was casually observed but it was surmised to be in the neighborhood of the air temperature. The following observations were taken during our stay in the Yapp Islands.

Locality (Yapp Island)	Date	Depth in meter					
St. 1		0 m	5 m	10 m	20 m	30 m	40 m
(In front of the goverment office building)	Oct. 30, 2.00 pm. 1929	32°.4C.	29°.9C.	29°.1C.		-	
St. II (Entrance of passage)	Oct. 30, 3.30 pm 1929	32°.0C.	-	29°.4C.	29°.0C.	29°.0C.	29°.0C.
St. IIII (where the coral growth was observed)	Jan 14, 2.00 pm. 1930	27°.3C.	27°.5C.	27°.5C.	27°.3C.	27°.1C	27°.1C.

At the Station III where the coral growth was observed, the oceanographical observations were made on January 14th 1930, with the results: transparency extending to 18 meters; water color, No. 3 (Forel scale); specific gravity, 1.02548 (28°.0 C.); and hydrogen-ion concentration, pH 8.25. The difference between high and low tide was about 1.5 meter in Yapp Island.

Palau Island which is situated on 7°.0 N.Lat. and 134°.5 E.Lon., consists of many islands, the largest one measuring about 310 sq. km. In gross appearance these islands may be regarded as being encircled by a barrier reef. The meteorological observations made by the government officers in Palau in 1927 are as follows:

The average monthly air temperature ranges from 27°.6 C. to 26°.6 C.

TABLE 1. Meteorological observation at Yapp Island in 1927.

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Mean
Atmos. press. mm.	756.3	757.5	756.9	756.6	756.8	757.3	756.1	755.9	757.4	756.7	756.6	757.0	756.7
Air temp. Mean °C.	26.1	25.9	26.3	26.1	26.4	26.2	25.5	25.1	25.7	25.5	26.3	25.5	26.0
Air temp. Maxim. °C.	29.1	28.8	29.6	30.7	30.5	30.9	31.2	30.1	31.8	31.1	30.1	29.5	31.8
Air temp. Minim. °C.	24.9	24.8	25.4	25.4	25.1	24.6	24.6	24.1	25.0	24.7	25.0	25.3	24.1
Humidity %	86.3	86.2	83.8	84.8	88.9	91.4	92.0	92.0	89.2	91.6	89.2	86.3	88.5
Wind	ENE	,,	,,	,,	,,	,,	WSW	,,	W	NNE	NF	,,	ENE
Volum of rain mm^3.	126.9	331.2	90.3	123.3	250.0	243.4	640.1	649.2	193.9	216.5	334.8	328.1	3527.8

TABLE 2. Meteorological observation at Palau Island in 1927.

	Janu.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Mean
Atmos. press. mm.	755.5	757.1	756.1	756.1	756.0	756.8	756.1	756.0	756.9	756.5	755.9	756.2	756.3
Air temp. Mean °C.	26.6	26.9	27.1	27.2	27.1	27.0	26.6	26.8	27.2	27.0	27.6	27.1	27.0
Air temp. Maxim. °C.	30.6	31.2	30.9	32.2	32.2	31.5	31.5	31.1	32.5	31.7	31.9	31.5	32.5
Air temp. Minim. °C	22.5	23.6	22.7	22.5	22.3	22.5	22.1	22.0	22.9	22.3	23.9	22.9	22.0
Humidity %	85.1	80.9	79.3	77.3	81.7	81.7	83.3	79.8	79.3	80.9	[illegible]	80.8	80.8
Wind	ENE	NE	ENE	SE	ESE	W	,,	SSW	N	W	NNE	NE	W
Volume of rain mm^3.	384.4	144.4	205.5	259.3	656.5	343.5	833.3	347.0	290.1	415.4	118.8	290.2	4378.4

and showing an average of 27°.0 C. The maximum and minimum air temperatures through the year were 32°.5 C. and 22°.0 C. respectively.

2) STATION OF OBSERVATION AND METHOD OF MEASUREMENT

Yapp Island. In Yapp Island the observations were carried out at two different stations.

Station A.—This station was fixed at a point about one mile off the shore of the municipal Hall. The depth was about 2 meters at high tide and 0.5 meters at low tide. Its bottom mainly consists of coral sands. About this station many species of reef building corals are found together with such animals as *Alcyonacea*, star fish, Gastropod molluscs, together with calcareous algae. The observation of the growth was made on 8 species of branching corals, 2 species of massive corals and 2 species of mush-room corals (*Fungia*).

Station B.—This station is situated about 1.5 miles off the shore of Maki, with the water depth being about 3.0 meters at high tide and 1.5 meters at low tide. At this station the fauna of the corals differed from those of the former station, not only destituting of *Fungia* but yields only about 6-7 species. The growth was directly observed on 2 species of both branching and massive corals, which were growing on the bottom.

Palau Island. In the Palau Island the station was centered in front of the Madarai pier of the Coloru Island. This station is a very quiet place, protected from wind and being inhabited by several corals such as branching forms, massive forms and *Fungia*. The growth was observed on seven species of *Fungia* and one species of massive corals.

METHOD

For the purpose of determining the growth of corals, it is necessary to select a specimen of definite stage, and also to fix firmly, wonder to prevent the loss by strong wave or current action. VAUGHAN (1915) used circular cement blocks of 8 inches in diameter, each with a hole in the center, upon which young corals attached themselves. We, however, chose the stem of a Teriha-tree, which is about one meter in length, having a section area of 10 sq. cm., upon which six small holes were made in order to insert the corals. Two wooden pieces measuring about 0.5 meters each, were attached to both ends of the wood, to which a heavy stone was tied in order to sink it into the sea for desired periods. In order to estimate the growth rate of the corals, the length, thickness, weight, volume, and

the number of branches were observed. In both islands the observations were made during the period from October to March but the annual growth of the corals was calculated from the assumption that in these localities the growth rate would be almost homogeneous as the environmental conditions are nearly constant throughout the year.

RESULTS

1. *Acropora abrotanoides* (LAM)?

This most common branching coral attains a height of about 2 meters in natural condition in Yapp island. The observations were made at Station A at Yapp island, for a period of 92 days, from Oct. 23rd to Jan. 24th 1930. The results obtained from six individuals are given in Table 3.

TABLE 3. *Acropora abrotanoides* (LAM)?

No.	Initial size			Period of growth	Increase for period			Annual increase	
	Length	Weight	Branch		Length	Weight	Branch	Length	Weight
	mm.	g.		days	mm.	%		mm.	%
1	120.0	126.0	7	92	39.0	7.3	3	156.0	29.2
2	111.0	125.0	9	92	29.0	14.9	0	116.0	59.6
3	95.0	68.0	4	92	26.0	7.9	0	104.0	31.6
4	92.0	64.0	4	92	32.0	10.0	0	128.0	40.0
5	84.0	37.0	2	92	33.0	18.9	0	132.0	75.6
6	68.0	29.0	1	92	29.0	17.2	0	116.0	68.8
Mean					31.3	12.7		125.3	50.8

From the above table we notice that the annual increase of length was 125.3 mm. in average while that of weight was 50.8% in average. At station B similar observations were made on 10 individuals for a period of 102 days, from Nov. 19th to Feb. (28th) 1930. During the observation 3 colonies died. The results obtained from the remaining 7 individuals are given in Table 4.

The annual increase of length was 101.7 mm. in average and that of the weight was 65% in average, showing slightly better growth at Station A.

2. *Acropora pulchra* (BROOK).

Observation of this species was made at station A. Like the former this species is found in shallow water, but differs in, its exhibiting a light brown color and possessing very loosely arranged calcarious skeletons. In natural condition its height reaches 1 meter or more. The results of the observations which were made on six colonies during the period from Oct.

TABLE 4. *Acropora abrotanoides* (LAM)?

No.	Initial size			Period of growth	Increase for period			Annual increase	
	Length	Weight	Branch		Length	Weight	Branch	Length	Weight
	mm.	g.		days	mm.	%		mm.	%
1	307.0	252.0	6	102	23.0	9.12	8	82.11	32.55
2	306.0	153.0	5	,,	3.0	9.01	3	10.71	33.16
3	290.0	503.0	14	,,	44.0	15.90	5	157.00	56.76
4	275.0	442.0	6	,,	28.0	8.59	0	99.96	30.66
5	265.0	685.0	17	,,	38.5	2.77	1	137.44	9.89
6	230.0	125.0	6	,,	28.0	33.20	1	99.96	118.50
7	146.0	104.0	5	,,	35.0	12.90	2	124.90	46.05
Mean					28.5	13.07		101.72	46.65

23rd to Dec. 20th (1929), are shown in Table 5. The annual increase of length was 225.8 mm. in average. The increase in weight was not measured. The growth rate of this species was most rapid among all the corals employed in the present study.

TABLE 5. *Acropora pulchra* (BROOK).

No.	Initial size			Period of growth	Increase for period		Annual increase
	Length	Weight	Branch		Length	Branch	Length
	mm.	g.		days	mm.		mm.
1	163.0	90.0	16	60	41.0	4	246.0
2	147.0	53.0	13	,,	38.0	0	228.0
3	127.0	64.0	11	,,	35.0	1	215.0
4	95.0	32.0	6	,,	37.0	1	222.0
5	92.0	29.0	6	,,	36.0	4	210.0
6	92.0	32.0	10	,,	39.0	2	234.0
Mean					37.66		225.8

3. *Acropora digitifera* (DANA).

This species is abundantly found in Yapp Island. The mode of branching differs much from the above two species already described, by growing only inwards thus exhibiting a mush-room-like appearance. The diameter of a large colony was over 2 meters with a height of 0.5 meters. The body is light bluish violet in color and the skeleton is hard and heavy. The observation was made at Station A in Yapp Island during the period from Oct. 29th to Feb. 24th (1930), extending to 118 days. The results taken from six specimens are given in Table 6. The annual growth of length was 11.95 mm in average and that of weight was 128.0% in average.

TABLE 6. *Acropora digitifera* (DANA).

No.	Initial size			Period of growth	Increase for period			Annual increase	
	Length	Weight	Branch		Length	Weight	Branch	Length	Weight
	mm.	g.		days	mm.	%		mm.	%
1	112.5	37.0	5	118	3.0	42.7	1	9.3	132.37
2	104.5	40.0	5	"	5.0	63.0	4	15.0	195.30
3	85.0	26.5	4	"	3.0	45.6	1	9.3	141.30
4	85.0	30.0	5	"	4.0	40.0	3	12.4	124.00
5	82.0	64.0	8	"	3.2	27.3	6	9.9	84.63
6	80.0	38.5	7	"	5.6	29.0	6	16.4	91.76
Mean					3.97	41.3		11.95	128.00

During the period of 118 days the branches increased from one to 6.

At station B from Nov. 19th to Feb. 28th (1930), extending for a period of 102 days, similar observations were made on the same species represented by 10 individuals and the results are shown in Table 7. Among the ten specimens observed, one colony died during the experiment. The annual growth of length was 10.1 mm. in average and that of weight was 65% in average.

TABLE 7. *Acropora digitifera* (DANA).

No.	Initial size			Period of growth	Increase for period			Annual increase	
	Length	Weight	Branch		Length	Weight	Branch	Length	Weight
	mm.	g.		days	mm.	%		mm.	%
1	175.0	380.0	26	102	4.0	22.3	4	14.28	79.61
2	171.0	185.0	14	"	2.5	18.9	7	8.93	67.47
3	160.0	400.0	30	"	1.0	19.2	10	3.57	68.54
4	134.0	140.0	14	"	5.0	12.1	2	17.85	43.19
5	133.0	135.0	11	"	2.0	12.5	0	7.14	44.62
6	129.0	159.0	19	"	4.0	16.6	0	14.28	59.26
7	121.0	150.0	8	"	2.0	10.6	0	7.14	37.84
8	98.0	134.0	26	"	4.0	30.5	8	14.28	108.80
9	88.0	193.0	24	"	1.0	21.2	1	3.57	75.68
Mean					2.65	19.9		10.11	65.00

The results show that the growth was much faster in station A than in station B which concords well with the case of *Acropora abrotanoides* (LAM) ?. Even natural habitates, the growth of the reef corals in station A exceeded those of station B.

4. *Acropora polymorpha.*

This is another common species found in Yapp Island. The observation

TABLE 8. *Acropora polymorpha.*

No.	Initial size			Period of growth	Increase for period			Annual increase	
	Length	Weight	Branch		Length	Weight	Branch	Length	Weight
	mm.	g.		days	mm.	%		mm.	%
1	105.0	36.0	8	118	9.5	37.5	6	28.45	116.25
2	94.0	32.0	5	,,	12.0	37.5	9	37.20	116.25
3	92.0	22.0	6	,,	5.0	45.4	2	15.50	140.70
4	89.0	25.0	5	,,	12.0	37.5	9	37.20	116.25
5	88.0	30.0	7	,,	9.5	37.5	6	28.45	116.25
Mean					7.84	34.7		22.20	124.70

was made during the period from Oct. 24th to Feb. 24th (1930), at station A, and the results are given in the Table 8. One specimen died during the course of experiments. The annual growth length was 22.2 mm. in average and that of the weight was 136.6 mm. in average. Nine branches were added in one case and only one in another during the 118 days.

5. *Porites nigrescens* DANA var.

This common species in Yapp Island presents a greenish brown color and its branches are short and irregular. Usually the height of the specimen found in Yapp did not exceed 2 meters. The observation continued during the period from Oct. 30th to Jan. 24th (1930), using 6 colonies and the results are given in Table 9.

TABLE 9. *Porites nigrescens* DANA var.

No.	Initial size			Period of growth	Increase for period			Annual increase	
	Length	Weight	Branch		Length	Weight	Branch	Length	Weight
	mm.	g.		days	mm.	%		mm.	%
1	102.5	115.5	5	86	3.5	32.7	0	14.8	138.6
2	91.8	90.0	5	,,	1.7	11.1	0	7.2	47.1
3	85.0	67.5	3	,,	6.0	13.3	0	25.4	56.4
4	56.0	20.0	1	,,	6.0	50.0	0	25.4	212.0
5	56.0	18.0	1	,,	5.0	42.3	0	21.2	179.4
6	53.0	15.5	1	,,	4.0	38.7	0	16.9	162.8
Mean					4.41	31.3		18.5	132.5

The annual growth of height was 18.5 mm. in average and that of the weight was 132.5% in average. No branches increased during the experiment.

6. *Stylophora mordax* DANA.

This species was found abundantly at station B in Yapp Island and

not at station A. The body is somewhat cube-shaped and white in color. The diameter did not exceed 40 cm. in the specimens found in Yapp Island. Observations were made for a period from Nov. 28th to Feb. 28th (1930) at station B. One colony among the ten specimens examined died during the period of 102 days. The results obtained from nine specimens are shown in Table 10.

TABLE 10. *Stylophora mordax* DANA.

No.	Initial size		Period of growth	Increase for period		Annual increase	
	Length	Weight		Length	Weight	Length	Weight
	mm.	g.	days	mm.	%	mm.	%
1	172.0	1040.0	102	11.0	20.1	39.3	71.76
2	164.0	800.0	,,	6.0	19.5	21.4	69.61
3	160.0	587.0	,,	12.0	18.0	42.8	64.26
4	147.0	560.0	,,	11.0	12.8	39.3	45.70
5	141.0	730.0	,,	17.2	19.0	61.4	67.83
6	131.0	625.0	,,	11.0	13.8	39.3	49.27
7	131.0	310.0	,,	5.0	6.1	17.9	21.78
8	125.0	260.0	,,	3.0	19.1	10.7	68.19
9	121.0	356.0	,,	10.0	13.9	35.7	49.62
Mean	.			9.57	15.81	34.2	56.41

The annual increase of length was 31.2 mm. in average and that of weight was 56.4% in average.

7. *Pocillopora malokensis* VAUGHAN.

This coral is found abundantly in Yapp Island, and its size in natural condition is over 2 meters in diameter. The observations were made during the period from Oct. 30th to Feb. 24th at station A in Yapp Island. The results obtained from 6 individuals are given in Table 11.

TABLE 11. *Pocillopora malokensis* VAUGHAN.

No.	Initial size			Period of growth	Increase for period			Annual increase	
	Length	Weight	Branch		Length	Weight	Branch	Length	Weight
	mm.	g.		days	mm	%		mm.	%
1	94.0	98.0	7	117	2.0	23.6	1	6.24	73.64
2	83.0	31.0	1	,,	0	4.8	0	0	14.68
3	74.0	56.0	3	,,	3.2	17.8	0	9.99	55.54
4	72.0	45.0	3	,,	4.5	33.3	2	14.04	103.90
5	65.0	20.0	1	,,	2.0	16.5	0	6.24	51.48
6	63.5	23.4	2	,,	2.3	20.5	0	7.18	63.96
Mean					2.33	19.4		6.28	60.53

Curiously enough one of the colonies showed no growth in the course of the experiment. The annual increase of length was 6.28 mm. in average and that of weight was 60.53% in average.

8. *Porites convexa* (VERRILL).

This species is found abundantly even where the salinity of water is much decreased due to discharge of inland water. The observations were made during the period from Oct. 31st to Feb. 24th (1930) at station A, and the results obtained from four individuals are shown in Table 12.

TABLE 12. *Porites convexa* (VERRILL).

No.	Initial size			Period of growth	Increase for period			Annual increase	
	Length	Weight	Branch		Length	Weight	Branch	Length	Weight
	mm	g.		days	mm.	%		mm.	%
1	94.0	24.0	1	85	4.1	12.5	0	18.89	53.63
2	94.0	22.5	2	,,	5.0	33.3	0	21.45	142.86
3	80.0	24.0	4	,,	3.5	12.5	0	15.02	53.63
4	69.0	11.0	1	,,	5.0	27.2	0	21.45	116.69
Mean					4.47	21.4		19.2	91.7

Two colonies among the 6 died in the course of 85 days. The annual increase of length was 19.2 mm. in average and that of weight was 91.7% in average. No branches were added during the experiment.

9. *Porites lutea.*

This massive coral is found abundantly in Yapp Island. It is yellowish brown in color, and the size of a large colony exceeds 4 meters in diameter.

TABLE 13. *Porites lutea.*

No.	Initial size				Period of growth	Increase for period				Annual increase	
	Length	Breadth	Thickness	Weight		Length	Breadth	Thickness	Weight	Length	Weight
	mm.	mm.	mm.	g	days	mm.	mm	mm.	%	mm.	%
1	120.0	83.0	70.0	770.0	102	5.0	2.0	0	9.22	17.85	32.91
2	103.0	83.0	67.0	470.0	,,	1.5	0	1.2	7.65	5.36	27.31
3	101.0	81.0	71.0	500.0	,,	4.0	0	1.0	12.40	14.28	44.26
4	88.0	48.0	25.0	105.0	,,	0	2.0	5.0	13.30	0	47.48
5	81.0	55.0	38.0	130.0	,,	1.0	0	4.0	27.60	3.57	98.53
6	81.0	60.0	41.0	210.0	,,	1.0	2.0	0	15.70	3.57	62.47
7	80.0	61.0	44.0	238.0	,,	2.0	0	1.0	4.38	7.14	15.64
8	80.0	48.0	43.0	140.0	,,	1.0	6.0	3.0	28.50	3.57	101.74
9	80.0	45.0	38.0	130.0	,,	0	2.0	0.3	20.70	0	73.90
Mean						1.71			15.49	6.15	56.02

Observations were made during the period from Nov. 15th to Feb. 24th (1930) at station A in Yapp. The results obtained from nine individuals are given in Table 13. One out of the ten colonies used, died during the experimental course of 102 days. The annual increase of length was calculated to be 17.85 mm. in maximum. Two of the specimens showed no growth during the experiment, but the average growth was found to be 6.15 mm. The annual increase of weight was calculated to be 56.02% in average.

10. *Montipora vercosa* (LAM).

This massive coral is found less frequently in the station B off Maki in Yapp. The body exhibits a purplish coloration, and a large specimen often exceeds 2 meters in diameter. The observations were made during the period from Nov. 19th to Feb. 28th (1930) at station B and the results obtained from ten experiments are given in Table 14.

TABLE 14. *Montipora vercosa* (LAM).

No.	Initial size			Period of growth	Increase for period			Annual increase	
	Length	Breadth	Weight		Length	Breadth	Weight	Length	Weight
	mm.	mm.	g.	days	mm.	mm.	%	mm	%
1	220.0	75.0	1040.0	102	2.0	0	6.25	7.14	22.31
2	184.0	76.0	570.0	,,	2.2	0	6.49	7.85	23.17
3	171.0	66.0	640.0	,,	1.5	0	5.15	5.36	18.38
4	133.0	103.0	1340.0	,,	2.2	7.0	5.58	7.85	19.92
5	130.0	87.0	700.0	,,	2.0	2.0	9.85	7.14	32.09
6	130.0	80.0	724.0	,,	3.0	0.5	4.97	10.71	17.74
7	126.0	96.0	1255.0	,,	2.0	4.0	4.98	7.14	17.77
8	118.0	85.0	565.0	,,	2.0	3.0	5.13	7.14	18.31
9	103.0	62.0	148.0	,,	1.0	0	10.40	3.57	37.12
10	101.0	85.0	617.0	,,	1.0	2.0	5.87	3.57	20.95
Mean					1.89		6.48	6.75	22.98

The annual increase of length was 6.75 mm. in average and that of weight was 22.98% in average.

11. *Fungia fungites* var. *haimei* VERRILL.

Altogether 3 species of *Fungia* are found in Yapp Island. The specimens chosen for the present study were of mush-room shaped form, which alives on the coral rock or on another species of living corals. The observations were made during the period from Nov. 15th to Feb. 24th (1930) at station A. The results obtained from ten experiments are given in Table 15. The annual increase of length was 6.13 mm. in average and that of weight was 16.2% in average.

TABLE 15. *Fungia fungites* var. *haimei* VERRILL.

No.	Initial size		Period of growth	Increase for period		Annual increase	
	Length	Weight		Length	Weight	Length	Weight
	mm.	g.	days	mm.	%	mm.	%
1	161.0	720.0	102	1.3	2.77	4.64	9.89
2	138.0	500.0	,,	2.0	3.40	7.14	12.13
3	134.0	370.0	,,	2.0	5.26	7.14	18.77
4	119.0	273.0	,,	2.0	4.02	7.14	14.35
5	118.0	323.0	,,	2.0	4.64	7.14	16.56
6	118.0	328.0	,,	2.5	6.09	8.93	21.74
7	116.0	279.0	,,	0.5	3.94	1.79	14.10
8	112.0	252.0	,,	1.0	5.15	3.57	18.38
9	101.0	179.0	,,	3.0	5.58	10.71	19.92
Mean				1.63	4.08	6.13	16.20

12. *Fungia echinatus* (PALLAS).

Unlike the former species, this mush-room shaped coral is elliptical in shape. The observations were made during the period from Nov. 15th to Feb, 24th (1930), at station A of Yapp Island using 9 colonies. The results are shown in Table 16. One of these specimens died in the course of 102 days. The annual growth length was 16.86 mm. in average and that of weight was 52.12% in average. In the two species of *Fungia* above described the later grew faster than the former.

TABLE 16. *Fungia echinatus* (PALLAS).

No.	Initial size			Period of growth	Increase for period			Annual increase	
	Length	Branch	Weight		Length	Branch	Weight	Length	Weight
	mm.	mm.	g.	days	mm.	mm.	%	mm.	%
1	167.0	80.0	360.0	102	5.5	0	3.33	19.63	11.88
2	155.0	75.0	310.0	,,	5.0	0	6.25	17.85	22.31
3	150.0	78.0	220.0	,,	7.0	1.0	6.81	24.99	24.31
4	143.0	68.0	180.0	,,	2.0	6.0	20.80	7.14	74.25
5	140.0	67.0	180.0	,,	5.0	3.0	21.10	17.85	75.32
6	135.0	67.0	157.0	,,	2.0	0	7.64	7.14	27.27
7	130.0	61.0	170.0	,,	5.5	1.0	14.10	19.63	50.33
8	130.0	67.0	157.0	,,	2.6	0	25.40	9.28	90.62
9	126.5	63.0	150.0	,,	8.0	2.0	26.00	28.56	92.82
Mean					4.73		14.63	16.89	52.12

13. *Fungia* sp.

Observations were made during the period from Nov. 1st to Feb. 17th (1931) in Palau Island. The results obtained from 18 experiments are given in Table 17. The annual increase of length was 6.3 mm. in average.

TABLE 17. *Fungia* sp.

No.	Initial size: Length	Breadth	Thickness	Weight	Period of growth	Increase for period: Length	Breadth	Thickness	Weight	Annual increase: Length	Weight
	mm.	mm.	mm.	gr.	days	mm.	mm.	mm.	%	mm.	%
1	173.0	166.0	32.0	667.1	110	1.7	2.0	0	?	5.62	?
2	159.0	149.0	30.4	643.1	,,	0.4	0.3	3.3	?	1.32	?
3	155.0	143.5	33.5	556.2	,,	0	0	3.0	?	0	?
4	153.2	152.8	30.0	505.6	,,	2.0	0.2	2.0	?	6.62	?
5	150.0	144.0	28.0	461.5	,,	1.5	0	0	0.81	4.96	22.52
6	144.8	147.5	28.5	504.1	,,	0.2	2.1	0	1.92	0.66	6.36
7	144.3	140.0	31.5	475.2	,,	2.0	3.1	0	4.92	6.62	16.28
8	142.0	140.0	33.0	429.3	,,	1.2	1.0	6.2	0.39	3.97	1.29
9	141.5	134.0	49.0	404.7	,,	1.8	2.6	0	15.56	5.96	51.50
10	137.6	136.0	35.0	411.3	,,	1.0	1.5	0	5.30	3.31	17.57
11	136.0	131.0	29.5	393.3	,,	0.2	0.7	1.6	5.67	0.66	18.76
12	131.0	126.2	34.0	315.4	,,	4.5	5.4	0	16.09	14.98	53.25
13	127.7	123.5	32.5	357.5	,,	5.3	1.8	0.5	6.28	17.54	20.79
14	119.4	117.5	26.2	245.0	,,	2.8	5.3	1.8	9.10	9.27	30.18
15	97.8	91.0	22.0	143.3	,,	3.4	3.8	1.8	11.67	11.25	38.62
16	96.1	96.7	22.0	164.9	,,	0.4	2.3	0	6.18	1.32	20.45
17	89.0	88.0	25.0	127.2	,,	3.0	6.0	0	15.66	9.93	51.83
18	77.0	74.0	17.5	65.5	,,	1.0	0.6	0	6.92	3.31	22.90
Mean						1.90			8.07	6.30	26.30

Several specimens showed no increase in length, but showed an increase in thickness and also in the number of branches. The average annual growth of weight was 26.66%. In weighing the specimens the water contained between the septum of *Fungia* was not completly removed, so the figures given in the table are all approximate figures.

14. *Fungia actiniformis* QUOY & GAIMARD var. *palawensis* DODERLEIN.

This *Fungia* has many long tube-like tentacles, and thus may easily

TABLE 18. *Fungia actiniformis* QUOY & GAIMARD var. *palawensis* DODERLEIN.

No.	Depth.	Initial size: Length	Breadth	Thickness	Period of growth	Increase of period: Length	Breadth	Thickness	Annual increase: Length	
	m.	mm.	mm.	mm.	days	mm.	mm.	mm.	mm	
1	1	100.8	88.8	25.0	94	0.7	0	0.6	2.72	
2	,,	63.2	60.0	21.1	,,	0.7	0.2	0.2	2.72	
3	,,	48.0	47.0	16.0	,,	6.6	4.6	1.6	25.60	
4	3	99.0	90.6	20.4	,,	0	0	6.1	0	
5	,,	74.0	67.5	25.0	,,	1.0	3.0	0	3.88	
6	,,	51.5	49.0	17.0	,,	1.2	1.2	0.5	4.66	
Mean									6.59	

TABLE 19. *Fungia fungites* var. *haimei* VERRILL.

No.	Depth	Initial size					Period of growth	Increase for period					Annual increase		
		Length	Breadth	Thickness	Weight	Volume		Length	Breadth	Thickness	Weight	Volume	Length	Weight	Volume
	m.	mm.	mm.	mm.	g.	c.c.	days	mm.	mm.	mm.	%	c.c.	mm.	%	c.c.
1	1	111.0	113.0	26.8	216.9	122.1	94	2.9	0.6	0.5	4.60	3.03	11.25	17.85	11.75
2	3	114.0	103.5	31.0	309.4	118.0	,,	0.3	0.4	1.3	8.25	0.34	1.16	32.05	1.31
3	7	80.3	75.5	?	?	?	,,	1.2	0.5	?	?	?	4.66	?	?
Mean													5.69	24.90	6.53

TAFLE 20. *Fungia costulata* ORTMANN.

No.	Depth	Initial size					Period of growth	Increase for period					Annual increase		
		Length	Breadth	Thickness	Weight	Volume		Length	Breadth	Thickness	Weight	Volume	Length	Weight	Volume
	m.	mm.	mm.	mm.	g.	c.c.	days	mm.	mm.	mm.	%	c.c.	mm	%	c.c.
1	1	82.0	78.1	23.0	161.5	69.5	94	1.7	1.4	1.2	6.29	5.0	6.60	24.40	19.40
2	5	90.0	83.8	21.8	152.1	74.0	,,	0.3	1.4	0	3.42	0	1.16	13.26	0
3	5	78.0	74.6	22.5	120.9	50.2	,,	0.2	0	2.0	2.72	3.07	0.78	10.55	11.91
4	10	64.5	62.2	?	?	?	,,	0.5	0.3	?	?	?	1.94	?	?
Mean								0.7	1.0	1.1	4.14	2.69	2.70	16.07	10.43

be distinguished from the other closely related species. Observations were made during the period of 94 days, from Nov. 18th to Feb. 20th (1931) in Palau Island. The data obtained from 6 specimens are given in Table 18.

The average annual increase of length was 6.59 mm. There were some specimens which showed no increase in length during the course of 94 days. The increase of weight was not observed in this case. The growth rate of one specimen placed in surface water and another in a depth of 3 meters showed no difference in this respect.

15. *Fungia fungites* var. *haimei* VERRILL.

Observations were made during the period from Nov. 18th to Feb. 20th (1931) in Palau Island, and the results obtained from three specimens are given in Table 19. In this experiment three specimens were placed at different depths, namely: surface, 3 meter deep, and 7 meter deep. The difference of growth due to depth was not remarkable. The annual increase of length was 4.3 mm. in average and that of weight was 24.93% in average. The increase of volume in the two specimens were 11.75 and 1.31%, with an average value of 6.53%.

16. *Fungia costulata* ORTMANN.

Observations on four individuals were made during the period from Nov. 18th to Feb. 20th (1931) in Palau Island, and the results are given in Table 20. The annual increase of length was 2.7 mm. in average and that of weight was 16.07% in average. The annual increase of volume determined on two specimens was 10.44% in average.

17. *Fungia echinatus* (PALLAS).

The observations were made during the period from Nov. 1st to Feb. 17th (1931) in Palau Island, and the results obtained from 14 individuals are given in Table 21. The annual increase of length was 14.8 mm. in average and that of weight was 18.41% in average.

18. *Herpetolitha stricta* (DANA).

Observations were made during the period from Nov. 1st to Feb. 17th (1931) in Palau Island, and the results obtained from two individuals are shown in Table 22. The annual increase of length was 23.4 mm. in average and that of weight was 14.63% in average.

19. *Fungia paumotensis* STUTCHBURY.

The observations were made during the period of 110 days in Palau Island, and the results obtained from five individuals are shown in Table 23. The annual increase of length was 3.9 mm. in average and that of weight was 18.78% in average.

20. *Favia speciosa* (DANA).

TABLE 21. *Fungia echinatus* (PALLAS).

No.	Initial size				Period of growth	Increase for period				Annual increase	
	Length	Breadth	Thickness	Weight		Length	Breadth	Thickness	Weight	Length	Weight
	mm.	mm.	mm.	g.	days	mm.	mm.	mm.	%	mm.	%
1	261.0	99.2	32.6	784.6	110	3.5	0	6.0	2.19	11.59	5.69
2	240.5	102.3	45.0	824.1	,,	0.7	0	0	?	2.32	?
3	236.0	118.0	48.0	787.6	,,	0.8	1.3	1.0	7.84	2.45	25.95
4	232.0	102.6	29.2	740.6	,,	7.5	1.0	11.1	4.18	24.82	13.83
5	231.1	80.0	25.0	600.6	,,	5.9	1.5	3.3	8.00	19.53	26.48
6	231.0	104.6	35.6	755.4	,,	5.8	15.3	11.4	12.45	19.20	41.42
7	218.0	96.3	42.0	575.1	,,	4.5	2.1	2.2	7.68	14.90	25.42
8	214.5	91.8	43.2	637.7	,,	2.7	2.0	0	5.68	8.94	18.80
9	208.0	90.4	45.0	587.7	,,	0.3	0	0	1.85	0.99	6.12
10	201.5	87.8	33.8	433.4	,,	3.2	1.2	0	0.76	10.92	2.52
11	200.2	83.5	30.5	404.6	,,	4.5	1.0	1.1	6.27	14.90	20.75
12	199.0	83.8	29.5	492.0	,,	13.0	0	4.3	2.74	43.02	9.07
13	196.3	74.6	24.0	343.2	,,	4.7	2.4	0.5	5.76	15.56	19.07
14	175.3	84.0	33.4	414.7	,,	3.2	1.0	1.2	7.11	10.59	23.53
Mean						4.5	2.6	3.2		14.80	18.41

TABLE 22. *Herpetolitha stricta* (DANA).

No.	Initial size				Period of growth	Increase for period				Annual increase	
	Length	Breadth	Thickness	Weight		Length	Breadth	Thickness	Weight	Length	Weight
	mm.	mm.	mm.	g.	days	mm.	mm.	mm.	%	mm.	%
1	233.5	75.5	34.5	506.9	110	6.9	?	0	2.03	22.83	6.72
2	194.7	74.0	26.0	344.5	,,	7.3	1.8	1.2	6.81	24.16	22.54
Mean						7.1	1.8		4.42	23.40	14.63

TABLE 23. *Fungia paumatensis* STUTCHBURY.

No.	Initial size				Period of growth	Increase for period				Annual increase	
	Length	Breadth	Thickness	Weight		Length	Breadth	Thickness	Weight	Length	Weight
	mm.	mm.	mm.	mm.	days	mm.	mm.	mm.	%	mm.	%
1	125.0	73.0	25.8	260.9	110	1.5	0	0	?	4.96	?
2	120.7	81.6	32.5	330.5	,,	0.1	0	0	?	0.33	?
3	109.0	71.5	24.0	206.0	,,	1.8	0	0	?	5.96	?
4	103.5	75.0	27.0	229.4	,,	1.0	2.0	0	4.12	3.31	13.64
5	95.8	63.0	21.8	130.6	,,	1.7	1.7	1.0	7.23	5.62	23.93
Mean						1.2			5.52	3.90	18.78

This coral builds a large massive colony at Palau Island, and its diameter exceeds 3 meters. The observations were made during the period

from Nov. 14th to Feb. 19th (1931), and the results obtained from three colonies are given in Table 24. The annual increase of lengthwise diameter was 5.5 mm. in average and that of weight was 19.47% in average. The annual increase of volume obtained from two specimens was 25.68–145.5%, showing an average of 85.5%.

TABLE 24. *Favia speciosa* (DANA).

No.	Initial size				Period of growth	Increase for period				Annual increase	
	Length	Breadth	Height	Weight		Length	Breadth	Height	Weight	Length	Weight
	mm.	mm.	mm.	g.	days	mm	mm.	mm.	%	mm.	%
1	161.5	151.8	98.0	1981.6	97	2.0	1.7	0.9	2.71	7.52	10.15
2	142.5	119.0	65.0	888.2	,,	0.6	7.5	2.2	7.88	2.26	29.63
3	140.8	127.4	103.0	1379.3	,,	2.0	5.6	9.2	5.00	7.52	18.80
Mean						1.53	4.93	4.10	5.19	5.73	19.47

DISCUSSION

The coral growth with which we are dealing in the present paper means simply the growth of a colony and not that of an individual. The coral propagates in two ways, i.e. sexualy and asexualy. The growth of a colony is accomplished exclusively by budding or by asexual reproduction. So that the natural method of studying the growth rate of a colony should be, by counting the number of coral zooid (polyps) which propagates by budding. In the present work, however, the growth rate was measured simply by determining the increase of dimension, weights etc.

The growth of several corals which were already given are summarised in Table 25. As will be seen from the table, the growth rate of corals differs with the species, as well as with locality.

In Yapp Island, the growth of four species of *Acropora* were observed. The results show that *Acropora pulchra* (BROOK) showed an annual growth of 225.8 mm. in average, while in *Acropora digitifera* (DANA) it was only 11.95 mm. in length. Consequently, it follows that under an equal condition the former species grows about twenty times as fast as the later species. It seems interesting to note in this connection that the former possesses a very loose and light skeleton contrasted with the dense and heavy skeletons of the later.

Among the branching Porites, *Porites nigrescens* alone showed an annual increase 19.2 mm. in length and 142.86% in weight.

TABLE 25. Growth rate of reef building corals.

	Species	Period of growth	Average increase for period		Annual increase		No. of exp.	Locality
			Length	Weight	Length	Weight		
		days	mm.	%	mm.	%		
1	*Acropora abrotanoides* (LAM)	92	31.30	12.70	125.33	50.80	6	Yapp A
2	,, ,,	102	28.50	13.07	101.72	46.65	7	,, B
3	*Acropora pulchra* (BROOK)	60	37.66	?	225.80	?	6	,, A
4	*Acropora digitifera* (DANA)	118	3.97	41.30	11.95	128.00	6	,, A
5	,, ,,	102	2.65	19.90	9.48	65.00	9	,, B
6	*Acropora polymorpha*	118	7.83	34.70	22.20	124.70	5	,, A
7	*Porites nigrescens* DANA var.	86	4.41	31.30	18.50	132.50	6	,, A
8	*Stylophora mordax* DANA	102	9.57	15.81	34.20	56.44	9	,, B
9	*Pocillopora malokensis* VAUGHAN	117	2.33	19.40	6.28	60.54	6	,, A
10	*Porites convexa* (VERRILL)	85	4.47	21.40	19.20	91.70	4	,, A
11	*Porites lutea*	102	1.71	15.49	6.15	56.02	9	,, A
12	*Montipora vercosa* (LAM)	102	1.89	6.48	6.75	22.98	10	,, B
13	*Fungia fungites* var.	102	1.63	4.08	6.13	16.20	10	,, A
14	*Fungia echinatus* (PALLAS)	102	4.73	14.63	16.89	52.12	9	,, A
15	*Fungia* sp.	110	1.90	8.07	6.30	26.66	19	Palau
16	*Fungia actinoformis* Q & G.	94	2.00	?	7.80	?	6	,,
17	*Fungia fungites* var.	94	1.10	6.40	4.30	24.93	3	,,
18	*Fungia costulata* ORTMANN	94	0.70	4.14	2.70	16.07	4	,,
19	*Fungia echinatus* (PALLAS)	110	4.50	5.63	14.80	18.41	14	,,
20	*Herpetolitha stricta* (DANA)	110	7.10	4.42	23.40	14.63	2	,,
21	*Fungia paumatensis* S.	110	1.20	5.52	3.90	18.78	5	,,
22	*Favia speciosa* (DANA)	97	1.50	5.19	5.50	19.47	3	,,

ASAHINA (1931) found that *Acropora* sp. grew 220 mm. in length during the period from Dec. 1925 to June 1930 at Palau Island. From this, he estimated its annual growth to be 44 mm. in length.

Massive corals such as ***Porites***, or ***Montipora*** showed an annual increase of 6.2-7.6 mm. in length and 23-56% in weight. EDMONDSON (1929) studied and growth rate of the massive porites in Hawaii and reported that it increases 8 to 10 mm. in length, and about 60% in weight annually.

As regards the mush-room corals, observations were made on two species in Yapp Island, and on seven species at Palau Island. The results show that the annual increase of these corals was 23.4–2.7 mm. or 9.85 mm. in average length and that of weight was 23.47%. EDMONDSON (1929) states that free specimens of ***Fungia*** are able to grow from 5 to 6 mm.

in length annually.

Thus all the corals enlarge their colonies with a certain rate characteristic to each, though they can not grow unlimitedly, and the growth ceases after attaining a certain size. This relation just mentioned has already been observed by several authors, for instances by GUPPY (1889) and by VAUGHAN (1915).

The size of the massive corals found in Yapp Island was mostly under 5 meters in diameter. According to MAYOR (1924), however, some colonies of *Porites* on the Great Barrier Reef measure 20 feet in diameter, while in Samoa Island it never exceeds 6.9 feet in diameter.

The largest *Fungia* found in Yapp Island measured about 10 cm. in length.

It is likely that similar as other animals, the coral can not continue its growth in a constant rate, and in addition some of them may be killed by the change of environmental factors such as salinity, temperature, current, food etc.

Indeed it was found at Yapp Island that among 112 specimens representing 12 species observed eight species died during the course of about 100 days, thus giving an annual death rate of 25,9%. Generally speaking, the reef building corals live in the shallow sea water above 10 fathoms. FORSTER COOPER (1905) (from GARDINER. 1931) made a collection of corals in the Indian Ocean and found that by 23 dregings at the depths of 41–45 fathoms, only one coral was obtained, while at the depth of 16–20 fathoms 36 corals, including 16 genera, were obtained by 29 dregings.

We obtained 3 species of *Fungia* at the depth of 3 10 meters at Palau Island, but regarding its vertical distribution we unfortunately have no data at present. For the purpose of reference the previous records of coral growth obtained by various investigators are tabulated:

Pacific Ocean

Species	Annual Length	Growth Weight	Locality	Worker	Date
Montipora incognita	1 inch	(35.7 weeks)	Funafuti	FINCK	1904
Pocillopora vercosa	-	150%	,,	,,	,,
Pocippopora grandis	1 inch	(13.5 weeks)	,,	,,	,,
Porites limor	-	42.27%	,,	,,	,,
Acropora	30 mm.	501 gs	Samoa	MAYOR	1924
Pocillopora	23 ,,	272 ,,	,,	,,	,,
Branching porites	30 ,,	222 ,,	,,	,,	,,
Massive porites	17 ,,	?	,,	,,	,,

Species	Annual Length	Growth Weight	Locality	Worker	Date
Pavana	32 mm.	34 gs	Samoa	MAYOR	1924
Psammocora	14 "	85 "	"	"	"
Porites astroeoides	1.95 inch	---	Murray	"	1918
Symphayllia	1.88 "	--	"	"	"
Porites evermanni	11.6 mm.	54.9%	Hawaii	EDMONDSON	1929
Porites lobata	7.1 "	54.6 "	"	"	"
Porites compressa	10.8 "	90.7 "	"	"	"
Pocillopora meandrina	14.8 "	148.0 "	"	"	"
Pocillopora ligulata	14.5 "	137.5 "	"	"	"
Pocillopora cespitosa	13.9 "	103.5 "	"	"	"
Montipora vercosa	14.0 "	51.5 "	"	"	"
Stephanaria stellata	5.7 "	48.5 "	"	"	"
Cyphastrea ocellina	2.0 "	22.2 "	"	"	"
Pavona varians	-- "	40.0 "	"	"	"
Fungia scutarix	4.4 "	17.2 "	"	"	"
Leptastrea agassizi	2.8 "	26.0 "	"	"	"
Acropora sp.	44.0 "	-	Palau	ASAHINA	1931
Atlantic Ocean					
Orbicella annularis	0.36 inch		Havana	AGASSIZ	1890
Manicina areolata	0.14 "		"	"	"
Isophyllia dipisacea	0.36 "		"	"	"
Orbicella annularis	5.7 mm.	29.1%	Gording cray,	VAUGHAN	1915
Orbicella annularis	5.28 "	-	Dry Tartugas	"	"
Acropora palmata	• 24.4 "	231.8%	Bahama	"	"
Porites clavaria (branch)	20.45 "		Dry Tortugas	"	"
Porites asteorides	5.7 "	-	Fort Jefferson	"	"
Gorgonia flabellum	51.0 "	--	Dry Tortugas	CARY	1914
Plaxaura flexuosa	20.0 "		"	"	"
Indian Ocean					
Branching *Porites*	1.5 inch		Cocos	GUPPY	1884
Massive *Porites*	0.5-0.75 "		"	"	"
Branching *Madreopores*	3.7 "	-	"	WOOD JONES	1910
Massive form	23.6 mm.		Maldives	GARDINER	1903
Branching coral	35.0 "	-	"	"	"

VAUGHAN (1915) concluded that massive corals grow more rapidly in the Indian and Pacific Oceans than in the Atlantic. MAYOR (1924) made an important investigation on the coral reefs at Florida and Dry Tortugas in the Atlantic, and at Samoa in the Pacific, and found that "the rate of growth of reef building corals in the Pacific was about twice as rapid

as that of coresponding genera in the Atlantic, where there is much more precipitate of coral mud and less favorable food conditions."

EDMONDSON (1929) investigated the growth rate of reef building corals at Hawaii and states that, on the surface of the reef in Hawaii, the increase in height was less rapid than in the Indian and South Pacific Ocean. The growth rate in Hawaii approximates the rate recorded in the Bahama and Dry Tortugas.

VAUGHAN (1916) and EDMONDSON (1928) studied the ecology of the coral and found that, favorable conditions for coral growth are found in the depth from surface to about 45 meters, firm bottom with no silty deposits, good circulation of water, good food supply, strong light, minimum temperature above 18°C., and salinity about 27–28‰ were said to be favorable to them.

The results of the present investigation indicate that the rate of the growth of reef corals not only differs with the species, but individual difference in the same species is also considerable. All we can state at the present is, that the growth rate of the corals living in the South Sea Islands is quite similar to those of the Hawaiian corals which were studied by EDMONDSON (1929).

SUMMARY

1. The growth rate of the reef corals were observed in two localities, i.e. Yapp and Palau Islands. In the Yapp Sea, eight species of branching forms, two species of massive forms and two species of *Fungia* were observed, while in Palau seven species of *Fungia* and one species of massive coral were observed.

2. Some species of *Acropora* were observed at two different stations in Yapp Island with the results that the growth rate is faster in the station where the other corals flourish better.

3. Observations were made on four different species belonging to genus *Acropora*, and it was found that the growth rate of *Acropora* differs with the species. The annual increase of the length of *Acropora pulchra* (BROOK) was 225.8 mm. while that of *Acropora digitifera* (DANA) was 11.95 mm. The differences here found appear to be due to the differences in the form of branching and in the composition of skeletons.

4. Massive corals such as *Porites*, *Montipora*, *Favia* showed an average annual increase of 6.13 mm. in length and 32.82 in weight.

5. The results obtained from seven species of mush-room corals (*Fungia*)

showed an annual increase of 9.58 mm. in length and 23.47% in weight.

6. In Yapp Island the growth rate of 112 individuals were observed during the period of about 100 days. Among those eight died during the course of experiment, giving a death rate of about 26% annually.

7. Whether or not the growth rate of *Fungia* is correlated with the depth of water was studied, but no definite results were obtained.

8. The rate of the growth of corals in Yapp and Palau Islands is similar to the Hawaiian corals.

9. Although different corals grow at different rates characteristic to each species, but after attaining a certain size the growth ceases in all the corals. The maximum size attainable within a given species may partly be due to the differences of environmental conditions.

(June 3rd, 1932).

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EXPLANATION OF PLATES.

PLATE XI.

Fig. 1. General view of the fixed stage of corals.

Fig. 2. *Acropora abrotanoides* (LAM)? $\times \frac{1}{3}$

Fig. 3 *Acropora pulchra* (BROOK). $\times \frac{1}{3}$

Fig. 4. *Acropora digitifera* (DANA). $\times \frac{1}{4}$

Fig. 5. *Acropora polymorpha.* $\times \frac{1}{3}$

Fig 6. *Porites convexa* (VERRILL). $\times \frac{1}{3}$

Fig 7. *Pocillopora malokensis* VAUGHAN. $\times \frac{1}{3}$

Fig 8. *Porites nigrescens* DANA var. $\times \frac{1}{3}$

PLATE XII.

Fig. 9. *Stylophora mordax* DANA. $\times \frac{1}{3}$

Fig. 10. *Porites lutea.* $\times \frac{1}{3}$

Fig. 11. *Montipora vercosa* (LAM). $\times \frac{1}{3}$

Fig 12. *Favia speciosa* (DANA). $\times \frac{10}{25}$

Fig. 13. *Fungia fungites* var. *haimei* VERRILL. $\times \frac{1}{3}$

Fig. 14. *Fungia echinatus* (PALLAS). $\times \frac{1}{5}$

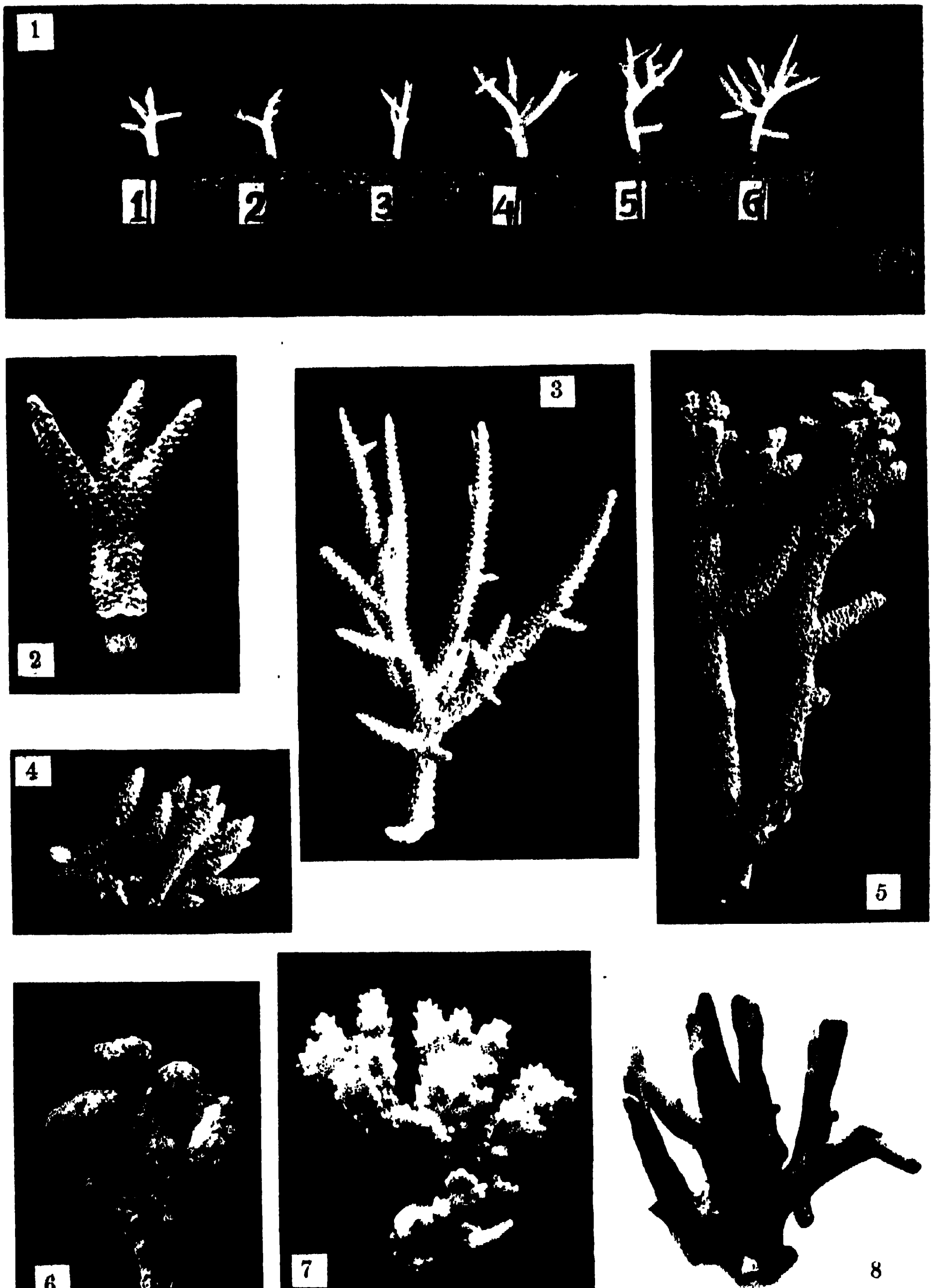
Fig. 15. *Fungia costulata* ORTMANN. $\times \frac{1}{3}$

Fig. 16. *Fungia* sp. $\times \frac{10}{24}$

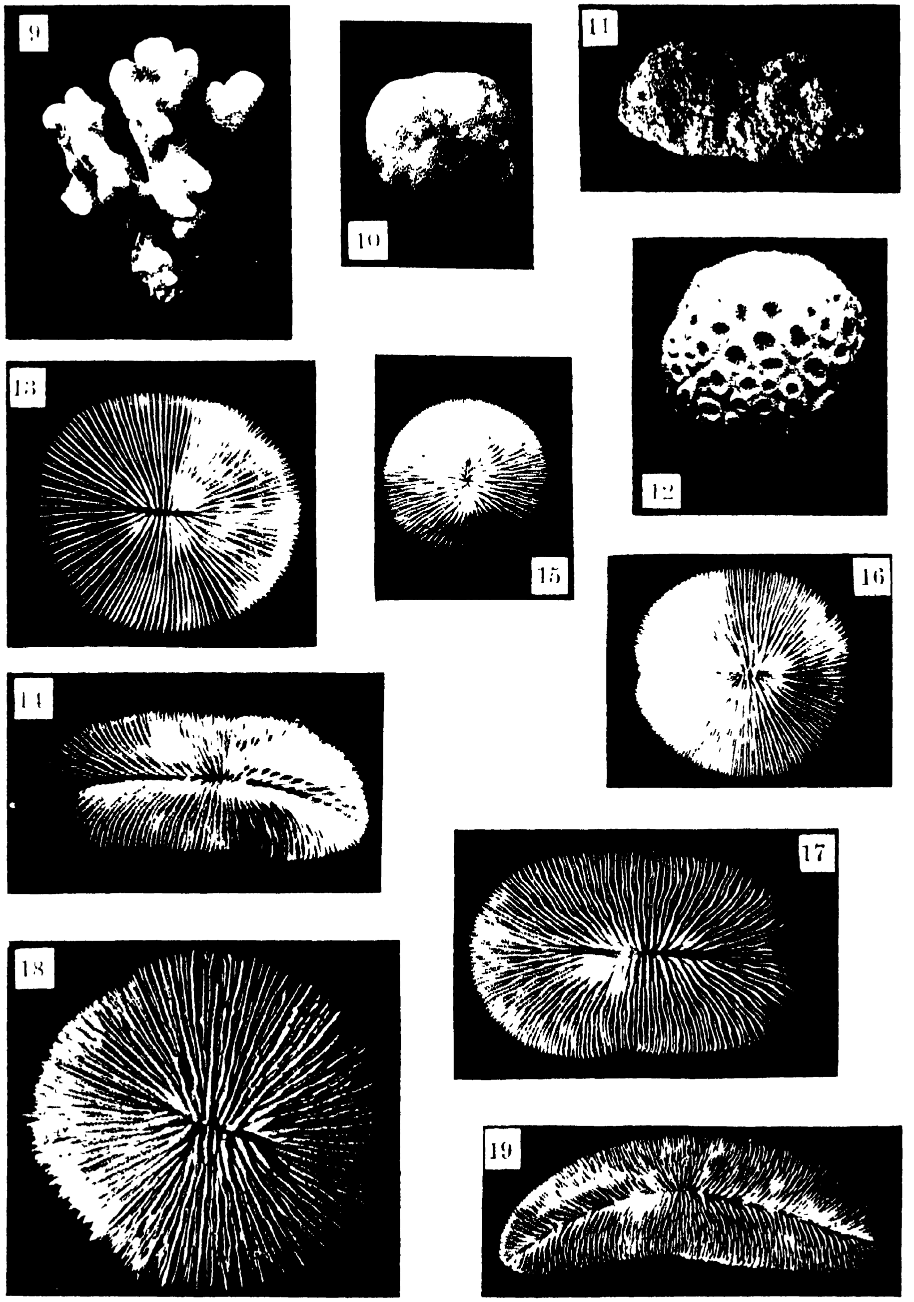
Fig. 17. *Fungia paumatensis* STUTCHBURY. $\times \frac{10}{26}$

Fig 18. *Fungia actiniformis* QUOY GAIMARD var. palawensis DODERLEIN. $\times \frac{10}{31}$

Fig. 19. *Herpetolitha stricta* (DANA). $\times \frac{1}{3}$



T. TAMURA and Y. HADA: Growth of Corals in South Sea Island.



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EFFECTS OF INORGANIC SALTS ON PHOTIC ORIENTATION IN *ALLOLOBOPHORA FOETIDA* (SAV.).
7. EFFECTS OF NaCl AND $MgCl_2$ OF DIFFERENT CONCENTRATIONS.

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ABSTRACT. In the present report, the effects of NaCl and of $MgCl_2$ on photic orientation in *Allolobophora foetida* are re-investigated in reference to different concentrations.

Serially different concentrations of the salts produce serially different effects on the photic orientation and mode of crawling.

All the factors by which alter the antagonistic relations between the functionings in the central nervous system are altered, viz. between positively and negatively orientating functioning, and between forward and backward functioning in crawling, cause a change in the tropism reaction.

So far as we have carried out experiments on the effects of inorganic salts on the photic orientation in *Allolobophora foetida*, among the salts used[1–6], we have been able to find one, viz. NaCl[1,5], which with a lapse of time causes a weakening of the positively orientating functioning in the ventral nerve cord and a strengthening of the negatively orientating functioning in the brain, and therefore a strengthening of the degree of negative orientation in all worms inclusively, and another, viz. $MgCl_2$[2,6], which, on the contrary, tends to cause a strengthening of positively orientating functioning in the ventral nerve cord, and a weakening of negatively orientating functioning in the brain, and, therefore, a weakening of the degree of negative orientation in all worms. We shall now proceed to study methodically the effects of different concentrations of NaCl and of $MgCl_2$.

1) $MgCl_2$, $CaCl_2$, NaCl, and KCl. Sci. Rep. Tôhoku Imp. Univ., 4th Ser., Vol. 3, No. 2, Pp. 151–177. Methods of experiments and of treating data are given in this paper.

2) $MgSO_4$, $FeSO_4$, Na_2SO_4, and K_2SO_4. *Ibid.*, Vol. 3, No. 3, Fasc. 1, Pp. 223–248.

3) $Mg(NO_3)_2$, $Ca(NO_3)_2$, $NaNO_3$, and KNO_3. *Ibid.*, Vol. 3, No. 3, Fasc. 2, Pp. 379–403.

4) NaI, KI, NaBr, and KBr. *Ibid.*, Vol. 3, No. 4, Fasc. 1, Pp. 647–663.

5) Sodium Salts, Na_2SO_4, $NaNO_3$, and NaCl. *Ibid.*, Vol. 5, No. 3, Pp. 467–483.

6) Magnesium Salts, $MgSO_4$, $Mg(NO_3)_2$, and $MgCl_2$. *Ibid.*, Vol. 5, No. 4, Pp. 669–689.

I. EFFECTS OF NaCl OF DIFFERENT CONCENTRATIONS.

The experiments were carried out in solutions at a temperature of 26.1°–26.7°C., July 15–27, 1929. In any one experiment, one of the normal solutions of NaCl, viz. 1/32, 1/16, 1/8, 1/4, 1/2, 3/4 or 1/1, was used for one of the durations of immersion, viz. 10, 20, 30 or 50 seconds. The control experiments or the experiments, in which the worms, both those operated on and those not operated on, were not immersed in any solution, were carried on at a temperature of 27.2°C., July 27, 1929.

A. Movements of Unoperated Worms.

50 worms were tested, individually, for one of the definite durations of immersion in one of the definite solutions.

After immersion in the 1/1 solution, the worms were seized with convulsions at the very beginning, with ejections of coelomic fluid after a lapse of about 3 seconds. They became inactive after 60 seconds and were dead in about 100 seconds.

In the case of the 3/4 solution, the worms were also seized with convulsions from the beginning of immersion, but the ejection of coelomic fluid began after about 10 seconds, the duration being somewhat prolonged. They became inactive after about 132 seconds and were dead in about 150 seconds.

On immersion in the 1/2 solution, the convulsions began after a lapse of about 24 seconds, and ejections of coelomic fluid in 38 seconds. The worms became inactive in about 420 seconds and were dead in 500 seconds.

On immersion in the 1/4 solution, the worms were seized with convulsions in 230 or more seconds, with an ejection of coelomic fluid in about 280 seconds. They were still active even after 700 seconds.

On being immersed in solutions weaker than 1/8, the worms neither went into convulsions nor was there any ejection of coelomic fluid even after 700 seconds, the duration of the period of attaining inactiveness being much prolonged.

Orientation.

The changes in the magnitude of the occupied angles are shown in Table 1. According to this table, in all the positive angles, the magnitude tends to decrease at first and then to increase with the prolongation of the duration of immersion, with the exception of the 5 cm. angles in the

case of the 1/1 solution in which it tends to increase successively. In spite of the general coincidence of the tendencies in the positive angles, several alterations were found in the negative angles. By using a method of graphic expression, however, out of 14 cases, including the 5 cm. and 10 cm. angles, we may select 6 or 7 in which the magnitude tends to increase with the prolongation of the duration of immersion, 2 in which it tends to decrease, the remaining 5 or 6 being cases in which it tends

TABLE 1.

Concentration	Duration of immersion in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
		Positive	Average	Negative	Positive	Average	Negative
$\frac{M}{32}$	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	74.88	95.18	110.30	76.36	111.88	125.52
	20	74.04	94.26	110.22	75.70	109.62	123.92
	30	76.60	96.36	109.76	75.68	114.08	128.40
	50	82.06	102.26	110.20	80.84	117.18	126.34
$\frac{M}{16}$	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	79.22	96.32	107.10	77.86	105.32	117.46
	20	75.56	95.50	109.94	75.90	104.06	118.16
	30	77.90	99.46	111.56	80.08	109.48	119.40
	50	78.26	105.60	117.34	80.72	116.82	126.10
$\frac{M}{8}$	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	73.90	94.86	110.96	75.18	110.68	125.20
	20	73.92	95.74	111.82	74.68	104.66	119.98
	30	72.10	97.70	115.60	71.84	105.66	123.82
	50	74.96	105.58	120.62	75.08	115.64	130.56
$\frac{M}{4}$	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	80.22	95.90	105.68	74.52	112.34	127.82
	20	73.94	92.06	108.12	72.46	107.04	124.58
	30	72.08	94.08	112.00	68.60	111.18	132.58
	50	81.88	105.80	113.92	71.56	114.52	132.96
$\frac{M}{2}$	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	80.50	100.76	110.26	77.14	112.30	125.16
	20	78.34	96.86	108.52	76.56	105.80	119.24
	30	79.20	98.52	109.32	75.82	106.80	120.98
	50	83.40	105.50	112.10	82.34	113.08	120.74
$\frac{3M}{4}$	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	78.00	99.20	111.20	77.02	111.70	124.68
	20	78.90	95.27	106.37	75.88	107.25	121.37
	30	79.56	100.74	111.18	78.64	110.56	121.92
	50	83.08	108.50	115.42	80.04	118.10	128.06
$\frac{M}{1}$	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	80.96	103.14	112.18	79.42	116.32	126.90
	20	82.53	105.10	112.57	79.71	117.69	127.98
	30	81.32	106.28	114.96	80.30	120.26	129.96
	50	86.54	113.10	116.56	84.28	120.34	126.06

TABLE 2.

Concentration	Duration of immersion in seconds	5 cm. angles			10 cm. angles		
		0°-80°	81°-99°	100°-180°	0°-80°	81°-99°	100°-180°
M/32	0	16	9	25	11	9	30
	10	21	8	21	15	8	27
	20	20	8	22	15	6	29
	30	20	8	22	12	8	30
	50	10	7	33	7	4	39
M/16	0	16	9	25	11	9	30
	10	18	10	22	15	7	28
	20	19	9	22	14	10	26
	30	16	6	28	14	4	32
	50	14	6	30	14	3	33
M/8	0	16	9	25	11	9	30
	10	18	7	25	14	8	28
	20	19	4	27	19	5	26
	30	20	6	24	18	6	26
	50	18	7	25	17	4	29
M/4	0	16	9	25	11	9	30
	10	16	9	25	18	5	27
	20	18	8	24	18	5	27
	30	18	8	24	17	5	28
	50	15	8	27	15	7	28
M/2	0	16	9	25	11	9	30
	10	17	8	25	13	8	29
	20	18	9	23	15	7	28
	30	18	7	25	14	8	28
	50	13	9	28	13	9	28
3M/4	0	16	9	25	11	9	30
	10	17	8	25	19	3	28
	20	18	10	22	18	10	22
	30	18	8	24	15	5	30
	50	11	12	27	14	5	31
M/1	0	16	9	25	11	9	30
	10	13	11	26	11	6	33
	20	13	9	28	11	4	35
	30	12	8	30	10	4	36
	50	10	6	34	8	5	37

to decrease at first and then to increase. Thus, the tendencies shown by the positive and negative angles, both those of 5 cm. and of 10 cm., coincide, from the point of view of the ultimate increase of the magnitude of the angles, of course, within the limits of experiment.

As a result, the average angles showed, in general agreement with the results found by the frequency distribution of the worms which is given in Table 2, that the degree of negative orientation tended to weaken at first and then to strengthen, with 2 exceptional cases found in the case

of the 1/1 solution in which it tended to strengthen continuously. Thus it may be stated that, as a whole, the tendency may possibly be influenced more strongly by the alteration of the degree of positive than by that of negative orientation.

TABLE 3.

Concentration	Duration of immersion in seconds	5 cm. angles: Forwards: Directly	5 cm. angles: Forwards: After posterior elongation	5 cm. angles: Backwards: Directly	5 cm. angles: Backwards: After anterior elongation	10 cm. angles: Forwards	10 cm. angles: Backwards	Returning	Winding
M/32	0	50	0	0	0	50	0	0	0
	10	49	0	1	0	49	1	3	2
	20	50	0	0	0	50	0	4	0
	30	49	0	1	0	49	1	1	0
	50	47	0	3	0	47	3	0	2
M/16	0	50	0	0	0	50	0	0	0
	10	49	0	1	0	49	1	2	1
	20	50	0	0	0	50	0	0	0
	30	48	0	2	0	48	2	1	2
	50	47	0	3	0	47	3	2	2
M/8	0	50	0	0	0	50	0	0	0
	10	47	0	3	0	48	2	0	2
	20	50	0	0	0	50	0	0	0
	30	48	0	2	0	48	2	1	0
	50	47	0	3	0	46	4	0	1
M/4	0	50	0	0	0	50	0	0	0
	10	49	0	1	0	49	1	2	1
	20	49	0	1	0	49	1	0	0
	30	50	0	0	0	50	0	2	0
	50	49	0	1	0	49	1	0	0
M/2	0	50	0	0	0	50	0	0	0
	10	47	0	3	0	47	3	0	0
	20	46	1	3	0	48	2	0	0
	30	50	0	0	0	50	0	0	1
	50	50	0	0	0	50	0	0	0
3M/4	0	50	0	0	0	50	0	0	0
	10	50	0	0	0	50	0	1	0
	20	50	0	0	0	50	0	0	0
	30	50	0	0	0	50	0	0	0
	50	50	0	0	0	50	0	0	0
M/1	0	50	0	0	0	50	0	0	0
	10	50	0	0	0	50	0	0	0
	20	50	0	0	0	50	0	0	0
	30	50	0	0	0	50	0	0	0
	50	50	0	0	0	50	0	0	0

Crawling.

The absolute numbers of forward and backward crawling, returning, and winding individuals are given together in Table 3. The results given in this table may show that in the case of the weaker solutions the frequencies of the backward crawling, returning, and winding movements tended to increase after immersion, while in the case of the stronger solutions such effects were not detectable.

B. Movements of Operated Worms.

25 worms were experimented on, individually, as in the case of those not operated on.

Orientation.

The changes in the magnitude of the occupied angles are shown in Table 4, and the frequency distribution of the worms is given in Table 5.

The 5 cm. angles in the case of the 1/32, and 1/8–3/4 solutions, and the 10 cm. angles in that of the 1/8–1/2 solutions, in the general coincidence of the results from both tables, show that the magnitude tended to decrease at first and then to increase with the prolongation of the duration of submersion.

The results from the frequency distribution after using the 1/16 solution may be cases of successive diminution. But in the case of both the 5 cm. and 10 cm. angles, the results from the positive, average, and negative angles show a general agreement in the tendency of the magnitude to decrease at first and then to increase.

In the case of the 1/32 and 3/4 solutions, the positive 10 cm. angles tended to decrease successively while the corresponding average and negative angles tended to decrease at first and then to increase. The results relatively to the frequency distribution, however, show a tendency which may be a case of successive increase. Consequently, even in these solutions it may be presumed that the positive 10 cm. angles tend ultimately to increase.

Thus, summarizing, we may now conclude that in the case of the 1/32–3/4 solutions the general tendency of the degree of positive orientation to be stronger at first and then to weaken has been demonstrated.

Finally, in the case of the 1/1 solution we are unable to find any coincidence not only in the results in Tables 4 and 5, but even in those

given by the positive, average, and negative angles. It rather seems to us that in this solution the worms show no notable alteration of the degree of positive orientation.

Moreover, it may be noted here that a serial alteration of tendencies due to different concentrations of the solution, which was difficult to detect in the case of the worms not operated on, may be shown somewhat clearly by the present set of experiments: that is a tendency, in the case of a

TABLE 4.

Concentration	Duration of immersion in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
		Positive	Average	Negative	Positive	Average	Negative
M/32	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	53.95	58.75	94.80	46.38	49.92	93.54
	20	52.44	55.04	92.60	44.76	48.64	93.88
	30	53.20	54.84	91.64	42.20	47.20	95.00
	50	54.60	57.20	92.60	37.40	51.04	103.64
M/16	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	60.60	61.20	90.60	11.80	41.80	90.00
	20	50.52	53.40	92.88	39.24	41.12	91.88
	30	53.60	56.72	93.12	39.48	44.56	95.08
	50	59.44	62.44	93.00	44.80	50.36	95.56
M/8	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	55.80	59.08	93.28	40.88	45.76	94.88
	20	52.40	53.52	91.12	39.80	39.92	90.12
	30	52.12	54.52	92.40	40.04	42.00	91.96
	50	58.72	63.56	94.84	42.96	49.68	96.72
M/4	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	55.24	58.88	93.64	45.68	50.92	95.24
	20	50.48	53.16	92.68	42.00	43.84	91.84
	30	52.32	53.92	91.60	44.36	16.48	92.12
	50	59.00	63.80	94.80	49.08	53. 2	94.04
M/2	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	54.12	60.64	96.52	10.76	47.36	96.60
	20	52.72	57.20	94.48	39.20	45.00	95.80
	30	56.20	61.60	95.40	41.36	46.48	95.12
	50	57.88	64.96	97.08	41.64	50.64	99.00
3M/4	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	58.76	62.52	93.76	12.64	48.40	95.76
	20	54.52	59.96	95.44	42.16	17.36	95.20
	30	56.68	63.60	96.92	40.10	49.40	99.00
	50	63.92	65.88	100.96	40.24	52.76	102.52
M/1	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	59.24	64.92	95.68	47.60	54.84	97.24
	20	61.20	65.96	94.76	43.36	51.48	98.12
	30	58.92	64.68	95.76	46.08	52.92	96.84
	50	60.44	67.48	97.04	49.36	55.20	95.84

TABLE 5.

Concentration	Duration of immersion in seconds	5 cm. angles			10 cm. angles		
		0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
$\frac{M}{32}$	0	15	6	4	19	2	4
	10	17	4	4	18	3	4
	20	17	4	4	19	2	4
	30	17	1	7	17	2	6
	50	16	1	8	16	1	8
$\frac{M}{16}$	0	15	6	4	19	2	4
	10	20	5	0	23	2	0
	20	21	1	3	21	2	2
	30	21	2	2	21	1	3
	50	20	3	2	22	0	3
$\frac{M}{8}$	0	15	6	4	19	2	4
	10	17	4	4	20	1	4
	20	19	5	1	21	4	0
	30	20	3	2	21	3	1
	50	15	4	6	18	2	5
$\frac{M}{4}$	0	15	6	4	19	2	4
	10	16	5	4	19	2	4
	20	18	4	3	21	2	2
	30	20	4	1	20	3	2
	50	18	2	5	20	1	4
$\frac{M}{2}$	0	15	6	4	19	2	4
	10	17	4	4	20	2	3
	20	20	2	3	22	0	3
	30	18	4	3	19	2	4
	50	16	6	3	18	2	5
$\frac{3M}{4}$	0	15	6	4	19	2	4
	10	18	5	2	19	2	4
	20	18	4	3	16	3	6
	30	15	5	5	15	2	8
	50	15	3	7	16	1	8
$\frac{M}{1}$	0	15	6	4	19	2	4
	10	14	6	5	18	3	4
	20	16	5	4	19	3	3
	30	15	5	5	20	2	3
	50	15	6	4	20	2	3

weak solution, of the degree of positive orientation to strengthen at first and then to weaken, altered to a tendency, of the degree of positive orientation to weaken successively, as the concentration increased.

Crawling.

From Table 6 we may infer as follows:

The frequency of backward crawling was increased after immersion in

the solutions of NaCl, and the movement may be more pronounced in a stronger solution than in a weaker.

The number of winding individuals did not show any special increase

TABLE 6.

Concentration	Duration of immersion in seconds	5 cm. angles: Forwards: Directly	5 cm. angles: Forwards: After posterior elongation	5 cm. angles: Backwards: Directly	5 cm. angles: Backwards: After anterior elongation	10 cm. angles: Forwards	10 cm. angles: Backwards	Returning	Winding
$\frac{M}{32}$	0	15	0	9	1	15	10	0	1
	10	10	1	13	1	11	14	0	0
	20	15	1	7	2	15	10	0	0
	30	11	0	14	0	10	15	0	1
	50	9	0	14	2	9	16	0	0
$\frac{M}{16}$	0	15	0	9	1	15	10	0	1
	10	13	0	9	3	13	12	0	0
	20	11	0	12	2	11	14	0	0
	30	13	0	11	1	13	12	0	0
	50	6	1	17	1	7	18	0	0
$\frac{M}{8}$	0	15	0	9	1	15	10	0	1
	10	12	1	12	0	13	12	0	0
	20	12	1	11	1	12	13	0	0
	30	9	0	16	0	9	16	0	0
	50	5	0	17	3	5	20	0	0
$\frac{M}{4}$	0	15	0	9	1	15	10	0	1
	10	4	1	19	1	5	20	0	1
	20	4	0	20	1	4	21	0	0
	30	6	0	17	2	6	19	0	1
	50	3	0	20	2	3	22	0	1
$\frac{M}{2}$	0	15	0	9	1	15	10	0	1
	10	9	0	16	0	9	16	0	0
	20	6	1	17	1	7	18	0	1
	30	6	0	16	3	6	19	0	3
	50	9	0	16	0	9	16	0	1
$\frac{3M}{4}$	0	15	0	9	1	15	10	0	1
	10	5	0	19	1	5	20	0	1
	20	6	0	17	2	6	19	0	0
	30	4	0	20	1	4	21	0	0
	50	9	0	15	1	9	16	0	0
$\frac{M}{1}$	0	15	0	9	1	15	10	0	1
	10	8	0	16	1	8	17	0	0
	20	6	0	18	1	6	19	0	0
	30	7	1	16	1	8	17	0	1
	50	6	0	18	1	6	19	0	1

even after immersion, especially in weak solutions.

No returning individuals were found.

C. Changes of Negativity in the Brain.

The magnitudes of *P*, *A*, and *N* are given together in Table 7, and from this table Figs. 1–7 were prepared to show the character of the changes. In these figures the tracings of the 5 cm. angles are denoted

TABLE 7.

Concentration	Duration of immersion in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
		P	*A*	*N*	*P*	*A*	*N*
M/32	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	58.75	95.18	126.43	49.92	111.88	151.96
	20	55.04	94.26	129.22	48.64	109.62	150.98
	30	54.84	96.36	131.52	47.20	114.08	156.88
	50	57.20	102.26	135.06	51.04	117.18	156.14
M/16	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	61.20	96.32	125.12	44.80	105.32	150.52
	20	53.40	95.50	132.10	41.12	104.06	152.94
	30	56.72	99.46	132.74	44.56	109.48	154.92
	50	62.44	105.60	133.16	50.36	116.82	156.46
M/8	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	59.08	94.86	125.78	45.76	110.68	154.92
	20	53.52	95.74	132.22	39.92	104.66	154.74
	30	54.52	97.70	133.18	42.00	105.66	153.66
	50	63.56	105.58	132.02	49.68	115.64	155.96
M/4	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	58.88	95.90	127.02	50.92	112.34	151.42
	20	53.16	92.06	128.90	43.84	107.04	153.20
	30	53.92	94.08	130.16	46.48	111.18	154.70
	50	63.80	105.80	132.00	53.12	114.52	151.40
M/2	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	60.64	100.76	130.12	47.36	112.30	154.94
	20	57.20	96.86	129.66	45.00	105.80	150.80
	30	61.60	98.52	126.92	46.48	106.80	150.32
	50	64.96	105.50	130.54	50.64	113.08	152.44
3M/4	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	62.52	99.20	126.68	48.40	111.70	153.30
	20	59.96	95.27	125.31	47.36	107.25	149.89
	30	63.60	100.74	127.14	49.40	110.56	151.16
	50	65.88	108.50	132.62	52.76	118.10	155.34
M/1	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	64.92	103.14	128.22	54.84	116.32	151.48
	20	65.96	105.10	129.14	51.48	117.69	156.21
	30	64.68	106.28	131.60	52.92	120.26	157.34
	50	67.48	113.10	135.62	55.20	120.34	155.14

by the broken lines, and those of the 10 cm. angles by the full lines.

According to Figs. 1–7, the changes of the magnitude of N or of the degree of negative orientation in the brain showed a general resemblance in all the solutions, in spite of the presence of many irregularities in detail among the results. To put it briefly, in the case of the 1/32, 1/16, 1/8, 3/4, and 1/1 solutions, the tendency may be stated to strengthen the negativity with the prolongation of the duration of immersion. In the case of the 1/4 and 1/2 solutions, the tendency in the case of the 5 cm. angles may also be the same, even though in the case of the 10 cm. angles a slight weakening of the tendency was apparent. Thus we may here summarize by saying that, within the limits of experiment, the solutions of NaCl tended to strengthen the negativity in the brain with increase of the period of submersion.

D. Changes in Crawling.

From Tables 3 and 6, we may infer the following as results of immersion:

On immersion of the specimens in a weaker solution, the strengthening of the backward functioning in crawling was caused by a relative weakening of the forward functioning in both the brain and the ventral nerve cord, while, on immersion in a stronger solution, it was caused by a relative weakening of the forward functioning in the ventral nerve cord, but not in that of the brain.

Fig. 1. NaCl/32

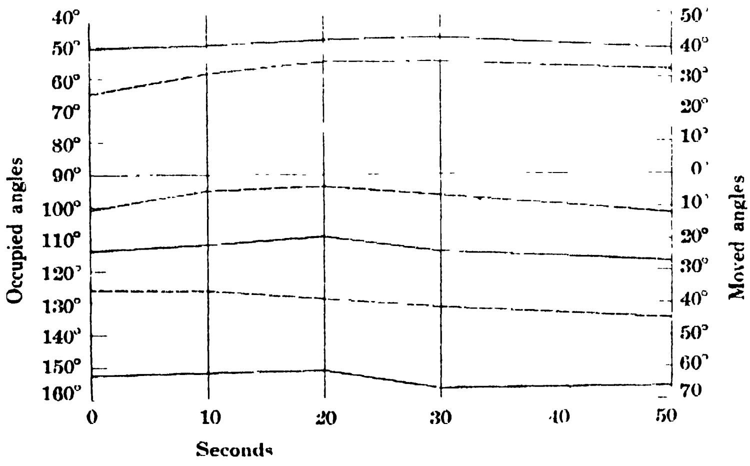


Fig. 2. NaCl/16

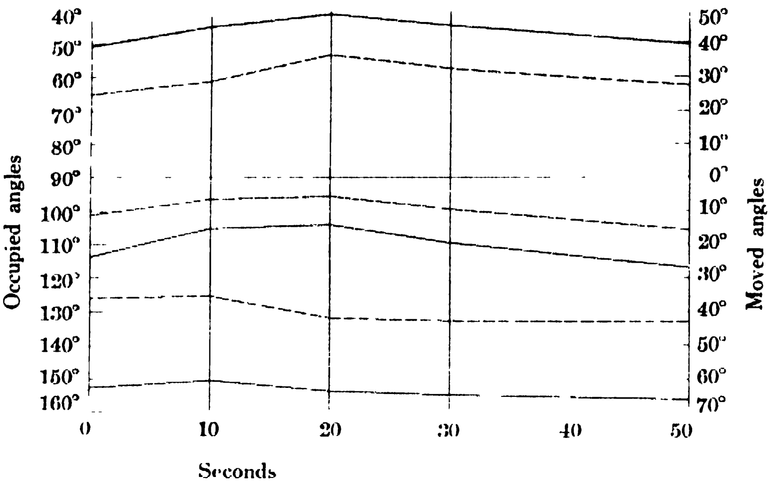


Fig. 3. NaCl/8

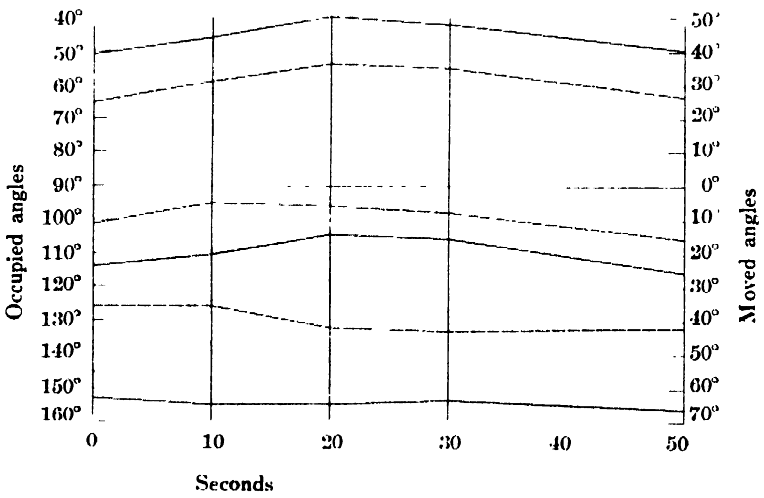


Fig. 4. NaCl/4

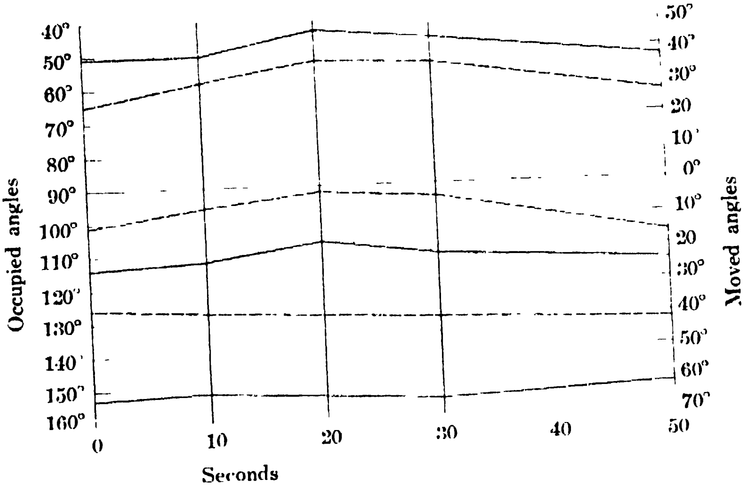


Fig. 5. NaCl/2

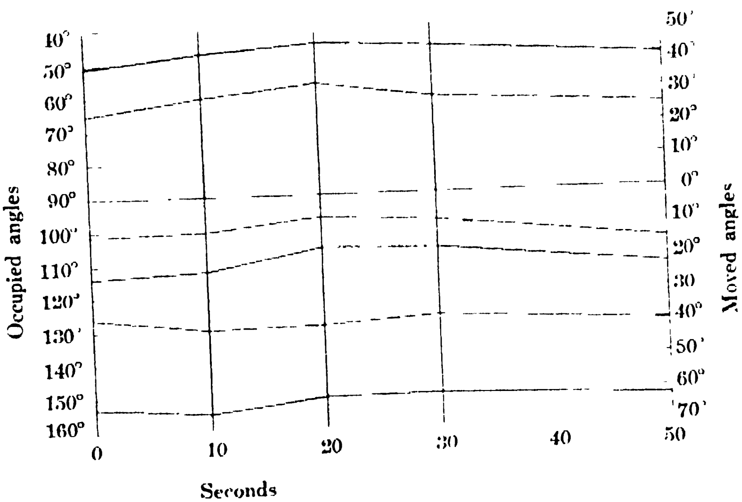


Fig. 6. 3 NaCl/4

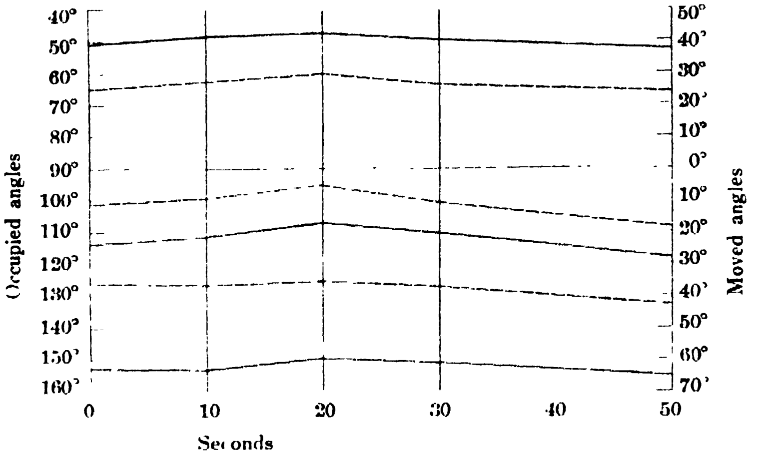
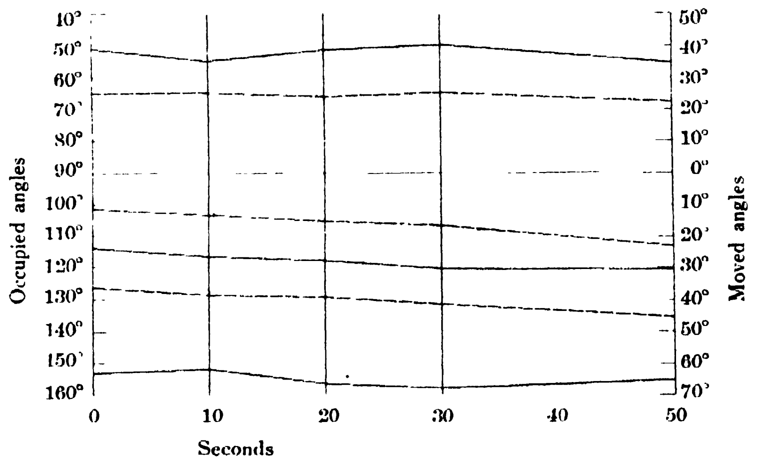


Fig. 7. NaCl/1



II. EFFECTS OF $MgCl_2$ OF DIFFERENT CONCENTRATIONS.

The experiments were carried on at a temperature of 26.7°–27.8°C., from July 28 to August 10, 1929. In any one experiment, a 1/32, 1/16, 1/8, 1/4, 1/2, 3/4 or 1/1 normal solution of $MgCl_2$ was used for one of the durations of immersion of 10, 20, 30, and 50 seconds. The control experiments were carried on at a temperature of 27.2°C., July 27, 1929.

A. Movements of Unoperated Worms.

50 worms were tested individually for one of the definite durations of immersion in one of the definite solutions.

After a lapse of 50-60 seconds from the beginning of immersion in the 1/1 solution, the worms were seized with convulsions. The ejection of coelomic fluid, however, rarely occurred, and when it did occur it was, in the majority of cases, previous to the convulsion. They became inactive after about 93 seconds and were dead in about 120 seconds.

In the case of the 3/4 solution, the worms were also seized with convulsions after about 80 seconds, but there was no ejection of coelomic fluid. They became inactive after about 113 seconds and died in about 150 seconds.

On immersion in the 1/2 solution, in the case of some of the worms convulsions were observed only after a lapse of more than 170 seconds, but no ejection of coelomic fluid occurred. They became inactive after about 280 seconds and were dead in about 350 seconds.

When immersed in solutions weaker than 1/4, the worms were neither seized with convulsions nor was there any ejection of coelemic fluid, and though they showed a tendency to become sluggish, they were still active even after a lapse of 500 seconds.

Orientation.

The changes in the magnitude of the occupied angles are shown in Table 8. According to this table, all the positive occupied angles, both 5 cm. and 10 cm., show a similar tendency to decrease in magnitude as the duration of immersion was prolonged, with the exception of the case of the 1/32 solution, in which the magnitude of the angles tended to decrease at first and then to increase. Nevertheless, in the negative occupied angles, the 5 cm. angles in the case of the 1/32-1/8 solutions and the 10 cm. angles in the case of the 1/32 and 1/16 solutions tended to increase in magnitude with the prolongation of the duration of immersion, while the 5 cm. angles in the case of the 1/4-1/1 solutions and the 10 cm. angles in the case of the 1/8-1/1 solutions tended to decrease.

As results, the ranges of the average occupied angles show a serial change of general tendencies with regard to the degree of negative orientation in coincidence with the results from the frequency distribution of the worms, which is given in Table 9: i. e. in the case of the 1/32 solution, the 5 cm. angles show a tendency to decrease at first, and then

TABLE 8.

Concentration	Duration of immersion in seconds	5 cm. angles in degrees: Positive	5 cm. angles in degrees: Average	5 cm. angles in degrees: Negative	10 cm. angles in degrees: Positive	10 cm. angles in degrees: Average	10 cm. angles in degrees: Negative
M/32	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	72.76	96.26	113.50	76.90	113.26	126.36
	20	75.70	97.82	112.12	80.06	118.14	128.08
	30	77.46	104.52	117.06	80.32	123.36	133.04
	50	78.32	106.36	118.04	80.66	123.96	133.30
M/16	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	78.66	104.04	115.38	79.24	117.84	128.60
	20	77.68	108.02	120.34	78.86	119.04	130.18
	30	79.92	104.50	114.58	79.68	115.98	126.30
	50	74.93	107.23	122.30	76.86	121.04	134.18
M/8	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	77.96	103.16	115.20	80.08	115.50	125.42
	20	74.92	106.44	121.52	79.68	112.44	122.76
	30	79.86	110.36	120.50	79.58	111.36	121.78
	50	75.94	107.44	121.50	75.00	107.30	122.30
M/4	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	73.88	92.20	108.32	75.80	98.26	112.46
	20	72.02	85.76	103.74	74.50	96.80	112.30
	30	70.56	84.04	103.48	71.38	99.31	117.93
	50	73.54	86.00	102.46	70.22	93.92	113.70
M/2	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	74.62	90.68	106.06	76.20	105.06	118.86
	20	72.58	85.64	103.06	76.32	92.20	105.88
	30	67.63	75.12	97.49	72.88	85.66	102.78
	50	69.62	80.04	100.42	64.68	79.16	104.48
3M/4	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	73.80	89.10	105.30	72.38	93.10	110.72
	20	71.92	81.46	99.54	71.50	88.88	107.38
	30	67.68	77.06	99.38	72.26	87.26	105.00
	50	67.20	75.70	98.50	74.92	86.40	101.48
M/1	0	79.96	101.06	111.10	81.22	113.90	122.68
	10	72.88	84.28	101.40	74.54	87.56	103.02
	20	68.68	80.78	102.10	62.02	76.76	104.74
	30	67.92	76.36	98.44	61.34	72.28	100.94
	50	66.02	66.46	90.44	60.54	62.12	91.58

to increase, in the degree of negative orientation with lapse of time, while the 10 cm. angles show a tendency to increase continuously, and in the case of the 3/4 and 1/1 solutions both the 5 cm. and 10 cm. angles show tendencies to a decrease in the degree of negative orientation, intermediate tendencies being shown in either the 5 cm. or 10 cm. angles, respectively, in the case of the 1/16-1/2 solutions.

TABLE 9.

Concentration	Duration of immersion in seconds	5 cm. angles 0°-80°	5 cm. angles 81°-99°	5 cm. angles 100°-180°	10 cm. angles 0°-80°	10 cm. angles 81°-99°	10 cm. angles 100°-180°
M/32	0	16	9	25	11	9	30
	10	20	6	24	14	4	32
	20	22	5	23	11	6	33
	30	15	8	27	11	5	34
	50	12	5	33	12	5	33
M/16	0	16	9	25	11	9	30
	10	16	7	27	11	8	31
	20	15	5	30	12	4	34
	30	13	9	28	12	7	31
	50	17	4	29	13	5	32
M/8	0	16	9	25	11	9	30
	10	16	11	23	12	10	28
	20	14	8	28	12	8	30
	30	14	5	31	13	5	32
	50	15	5	30	15	4	31
M/4	0	16	9	25	11	9	30
	10	19	8	23	17	7	26
	20	23	6	21	18	9	23
	30	21	9	20	18	5	27
	50	18	10	22	19	4	27
M/2	0	16	9	25	11	9	30
	10	18	11	21	18	4	28
	20	18	15	17	17	14	19
	30	22	10	18	22	10	18
	50	28	9	13	33	2	15
3M/4	0	16	9	25	11	9	30
	10	21	12	17	18	9	23
	20	27	11	12	21	10	19
	30	31	10	9	21	13	16
	50	29	12	9	18	16	16
M/1	0	16	9	25	11	9	30
	10	22	12	16	20	15	15
	20	24	8	18	27	6	17
	30	24	14	12	30	9	11
	50	26	11	13	31	9	10

Crawling.

The absolute numbers of forward and backward crawling, returning, and winding individuals are given in Table 10. In this table it may be seen that the backward crawling movement was slightly pronounced on submersion in a stronger solution, but less so in a weaker one, and that the returning and winding movements might be slightly intensified after immersion, but the relation to the concentration is not accurately shown.

TABLE 10.

Concentration	Duration of immersion in seconds	5 cm. angles: Forwards: Directly	5 cm. angles: Forwards: After posterior elongation	5 cm. angles: Backwards: Directly	5 cm. angles: Backwards: After anterior elongation	10 cm. angles: Forwards	10 cm. angles: Backward	Returning	Winding
M/32	0	50	0	0	0	50	0	0	0
	10	50	0	0	0	50	0	0	1
	20	50	0	0	0	50	0	0	0
	30	50	0	0	0	50	0	0	0
	50	50	0	0	0	50	0	0	0
M/16	0	50	0	0	0	50	0	0	0
	10	50	0	0	0	50	0	0	1
	20	50	0	0	0	50	0	0	1
	30	50	0	0	0	50	0	0	1
	50	50	0	0	0	50	0	0	1
M/8	0	50	0	0	0	50	0	0	0
	10	50	0	0	0	50	0	1	0
	20	50	0	0	0	50	0	0	0
	30	50	0	0	0	50	0	0	1
	50	50	0	0	0	50	0	0	0
M/4	0	50	0	0	0	50	0	0	0
	10	50	0	0	0	50	0	0	0
	20	50	0	0	0	50	0	0	0
	30	50	0	0	0	50	0	0	0
	50	50	0	0	0	50	0	0	1
M/2	0	50	0	0	0	50	0	0	0
	10	50	0	0	0	50	0	0	1
	20	50	0	0	0	50	0	0	0
	30	50	0	0	0	50	0	0	0
	50	50	0	0	0	50	0	0	1
3M/4	0	50	0	0	0	50	0	0	0
	10	50	0	0	0	50	0	0	0
	20	50	0	0	0	50	0	0	1
	30	49	0	1	0	49	1	0	0
	50	50	0	0	0	50	0	0	1
M/1	0	50	0	0	0	50	0	0	0
	10	50	0	0	0	50	0	0	1
	20	49	0	1	0	49	1	0	0
	30	48	0	2	0	48	2	0	2
	50	49	0	1	0	49	1	0	1

B. Movements of Operated Worms.

25 worms were tested individually as in the case of the worms not operated on.

Orientation.

The changes in the magnitude of the occupied angles are shown in Table 11, and the frequency distribution of the worms is given in Table 12 in absolute numbers. From these tables, in spite of the presence of many irregularities and of some dissimilarities in the tendencies to changes between the two tables, as in the case of the 5 cm. positive occupied

TABLE 11.

Concentration	Duration of immersion in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
		Positive	Average	Negative	Positive	Average	Negative
$\frac{M}{32}$	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	63.36	72.84	99.48	50.16	60.20	100.04
	20	64.92	77.76	102.84	52.96	67.68	104.72
	30	69.28	82.36	103.08	60.64	77.40	106.76
	50	70.76	81.20	100.41	63.96	78.00	104.04
$\frac{M}{16}$	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	56.48	63.76	97.28	46.84	53.68	96.84
	20	62.84	70.48	97.64	52.04	61.00	98.96
	30	66.68	75.20	98.52	45.04	55.72	100.68
	50	62.40	79.08	106.68	48.16	57.04	98.88
$\frac{M}{8}$	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	60.12	65.12	95.00	48.16	53.40	95.24
	20	62.52	66.04	93.52	49.32	54.48	95.16
	30	64.60	66.36	91.76	48.04	51.04	93.00
	50	65.72	67.16	91.44	47.44	57.44	100.00
$\frac{M}{4}$	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	53.76	58.32	94.56	48.80	53.24	94.44
	20	50.56	53.56	93.00	48.32	52.12	93.80
	30	54.52	56.68	92.16	46.04	54.80	98.76
	50	53.32	55.12	91.80	50.16	53.88	93.72
$\frac{M}{2}$	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	59.60	62.36	92.76	45.40	50.24	94.84
	20	59.96	62.04	92.08	46.28	48.32	92.04
	30	55.00	57.36	92.36	45.12	49.44	94.32
	50	57.24	63.56	96.32	49.84	56.72	96.88
$\frac{3M}{4}$	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	56.96	64.00	97.01	48.12	54.76	96.64
	20	56.64	62.56	95.92	46.48	49.44	92.96
	30	55.64	57.64	92.00	43.20	44.52	91.32
	50	49.28	53.76	94.48	40.36	44.36	91.00
$\frac{M}{1}$	0	59.00	65.24	96.24	45.16	51.04	95.88
	10	58.16	63.76	95.60	52.88	63.24	100.36
	20	60.88	62.80	91.92	56.56	59.24	92.68
	30	60.36	63.76	93.40	51.72	57.96	93.24
	50	57.88	58.88	91.00	44.96	46.68	91.72

angles in regard to the 1/16 and 1/8 solutions, we may generally consider the changes to be serial.

In the case of the 1/32 solution, the magnitudes of both the 5 cm. and 10 cm. positive occupied angles tend to increase with the prolongation of the duration of immersion, while in the 3/4 and 1/1 solutions they tend to decrease, even if an increase may occur at the beginning of immersion, intermediate tendencies to change having been found in the

TABLE 12.

Concentration	Duration of immersion in seconds	5 cm. angles			10 cm. angles		
		0°–80°	81°–95°	100°–180°	0°–80°	81°–99°	100°–180°
$\frac{M}{32}$	0	15	6	4	19	2	4
	10	15	5	5	17	4	4
	20	13	3	9	15	3	7
	30	12	3	10	14	1	10
	50	12	4	9	13	2	10
$\frac{M}{16}$	0	15	6	4	19	2	4
	10	19	2	4	19	2	4
	20	16	4	5	17	2	6
	30	16	5	4	17	3	5
	50	15	5	5	16	4	5
$\frac{M}{8}$	0	15	6	4	19	2	4
	10	16	5	4	19	2	4
	20	17	4	4	19	2	4
	30	20	3	2	17	4	4
	50	16	2	7	17	2	6
$\frac{M}{4}$	0	15	6	4	19	2	4
	10	17	2	6	19	2	4
	20	19	3	3	20	2	3
	30	19	3	3	19	2	4
	50	17	3	5	20	2	3
$\frac{M}{2}$	0	15	6	4	19	2	4
	10	20	3	2	21	3	1
	20	19	5	1	20	4	1
	30	20	2	3	21	0	4
	50	16	4	5	16	5	4
$\frac{3M}{4}$	0	15	6	4	19	2	4
	10	16	2	7	18	4	3
	20	17	4	4	19	4	2
	30	18	5	2	21	3	1
	50	18	4	3	20	3	2
$\frac{M}{1}$	0	15	6	4	19	2	4
	10	16	5	4	16	1	8
	20	17	6	2	17	6	2
	30	17	6	2	17	5	3
	50	17	7	1	21	2	2

case of the remaining solutions,—i. e. the 1/16, 1/8, 1/4, and 1/2 solutions.

In the case of the 1/32 and the 1/16 solutions, the magnitude of the 5 cm. negative occupied angles tended to increase with the increase in the number of seconds of immersion, while in that of the 1/8–1/1 solutions it tended to decrease; and in the case of the 1/32–1/8 solutions, the 10 cm. negative occupied angles tended to increase, while in that of the 1/4–1/1 solutions they tended to decrease.

As results, in support of Table 12, in the 1/32 solution, the 5 cm. average occupied angles tended to show a strengthening of the degree of negative orientation as the duration of submersion was prolonged, while in the case of the 3/4 and 1/1 solutions they tended to show a weakening, the intermediate tendencies to changes having been shown in that of the 1/16–1/2 solutions; and in the case of the 1/32–1/8 solutions, the 10 cm. average occupied angles tend to show a strengthening of the degree of negative orientation, while in that of the 3/4 and 1/1 solutions they showed a strengthening at first and then a weakening, intermediate tendencies having been shown in the case of the 1/4 and 1/2 solutions.

Crawling.

In Table 13 the following tendencies may be observed:

The number of backward crawling individuals tended to decrease when the worms were immersed in the weaker solutions of $MgCl_2$, but in the

TABLE 13.

Concentration	Duration of immersion in seconds	5 cm. angles: Forwards: Directly	5 cm. angles: Forwards: After posterior elongation	5 cm. angles: Backwards: Directly	5 cm. angles: Backwards: After anterior elongation	10 cm. angles: Forwards	10 cm. angles: Backwards	Returning	Winding
M/32	0	15	0	9	1	15	10	0	1
	10	19	0	5	1	18	7	0	1
	20	16	0	8	1	16	9	0	0
	30	21	1	2	1	22	3	0	0
	50	17	0	7	1	17	8	0	0
M/16	0	15	0	9	1	15	10	0	1
	10	23	0	2	0	23	2	0	1
	20	19	0	5	1	19	6	0	0
	30	22	0	1	2	21	4	0	0
	50	22	0	2	1	22	3	0	0

Concentration	Duration of immersion in seconds	5 cm. angles: Forwards: Directly	5 cm. angles: Forwards: After posterior elongation	5 cm. angles: Backwards: Directly	5 cm. angles: Backwards: After anterior elongation	10 cm. angles: Forwards	10 cm. angles: Backwards	Returning	Winding
M/8	0	15	0	9	1	15	10	0	1
	10	22	0	2	1	22	3	0	2
	20	17	0	4	4	17	8	0	0
	30	20	0	3	2	20	5	0	0
	50	16	0	8	1	15	10	0	0
M/4	0	15	0	9	1	15	10	0	1
	10	21	0	4	0	21	4	0	1
	20	20	0	4	1	20	5	0	0
	30	23	0	1	1	21	4	0	0
	50	23	0	2	0	23	2	0	0
M/2	0	15	0	9	1	15	10	0	1
	10	11	0	14	0	11	14	0	1
	20	11	0	13	1	11	14	0	0
	30	14	0	11	0	14	11	0	0
	50	11	0	11	3	10	15	0	1
3M/4	0	15	0	9	1	15	10	0	1
	10	11	0	14	0	11	14	0	0
	20	10	0	15	0	10	15	0	0
	30	12	0	13	0	12	13	0	1
	50	14	0	7	4	14	11	0	1
M/1	0	15	0	9	1	15	10	0	1
	10	13	0	10	2	11	14	0	0
	20	19	0	4	2	18	7	0	0
	30	22	0	3	0	22	3	0	1
	50	16	0	5	4	15	10	0	1

case of the stronger solutions it tended to increase.

No returning individuals were found.

The winding mevement did not appear to be intensified during immersion in the solutions.

C. Changes of Negativity in the Brain.

The magnitudes of *P*, *A*, and *N*, are given together in Table 14, and from this table **Figs.** 8–14 were plotted to ascertain the nature of these changes. In these **figures** the tracings of the 5 cm. angles are denoted by the broken lines and these of the 10 cm. angles by the full lines.

According to Figs. 8–14, in all the cases the magnitude of *N* or the degree of negative orientation in the brain tended to decrease with the

TABLE 14.

Concentration	Duration of immersion in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
		P	*A*	*N*	*P*	*A*	*N*
M/32	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	72.84	96.26	113.42	60.20	113.26	143.06
	20	77.76	97.82	110.06	67.68	118.14	140.46
	30	82.36	104.52	112.16	77.40	123.36	135.96
	50	81.20	106.36	115.16	78.00	123.96	135.96
M/16	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	63.76	104.04	130.28	53.68	117.84	154.16
	20	70.48	108.02	127.54	61.00	119.04	148.04
	30	75.20	104.50	119.30	55.72	115.98	150.26
	50	79.08	107.23	118.15	57.01	121.04	154.00
M/8	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	65.12	103.16	128.04	53.40	115.50	152.10
	20	66.04	106.44	130.40	54.48	112.44	147.96
	30	66.36	110.36	134.00	51.04	111.36	150.32
	50	67.16	107.44	130.28	57.44	107.30	139.86
M/4	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	58.32	92.20	123.88	53.24	98.26	135.02
	20	53.56	85.76	122.20	52.12	96.80	134.68
	30	56.68	84.04	117.36	54.80	99.31	134.51
	50	55.12	86.00	120.88	53.88	93.92	130.04
M/2	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	62.36	90.68	118.32	50.24	105.06	144.82
	20	62.04	85.64	113.60	48.32	92.20	133.88
	30	57.36	75.12	107.76	49.44	85.66	126.22
	50	63.56	80.04	106.48	56.72	79.16	112.44
3M/4	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	64.00	89.10	115.10	54.76	93.10	128.34
	20	62.56	81.46	108.90	49.44	88.88	129.44
	30	57.64	77.06	109.42	44.52	87.26	132.74
	50	53.76	75.70	111.94	44.36	86.40	132.04
M/1	0	65.24	101.06	125.82	51.04	113.90	152.86
	10	63.76	84.28	110.52	63.24	87.56	114.32
	20	62.80	80.78	107.98	59.24	76.76	107.52
	30	63.76	76.36	102.60	57.96	72.28	104.32
	50	58.88	66.46	97.58	46.68	62.12	105.44

prolongation of the duration of immersion with only two exceptional cases, and even in these latter the corresponding sets of the angles show also a decreasing tendency, i. e. the 10 cm. angles in the case of the 1/8 solution tend to decrease, although the 5 cm. angles in that of the same solution tend to increase; and the 5 cm. angles in the case of the 1/16 solution tend to decrease, although the 10 cm. angles in that of the same solution show a tendency but obscure. From the twelve cases out of

Fig. 8. $MgCl_2/32$

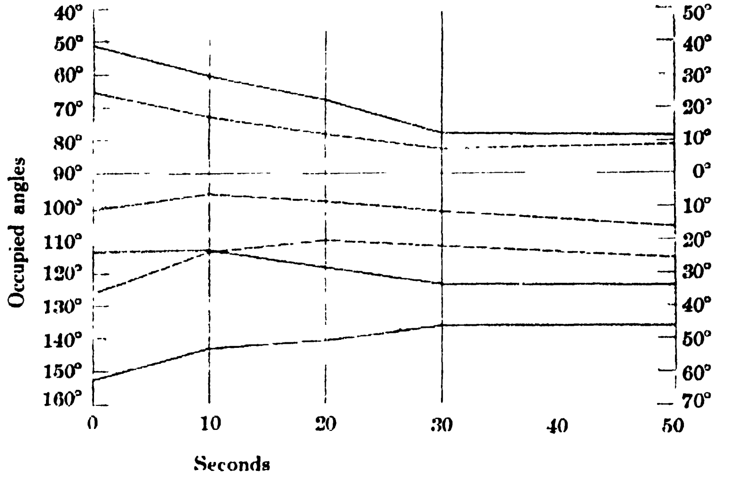


Fig 9. $MgCl_2/16$

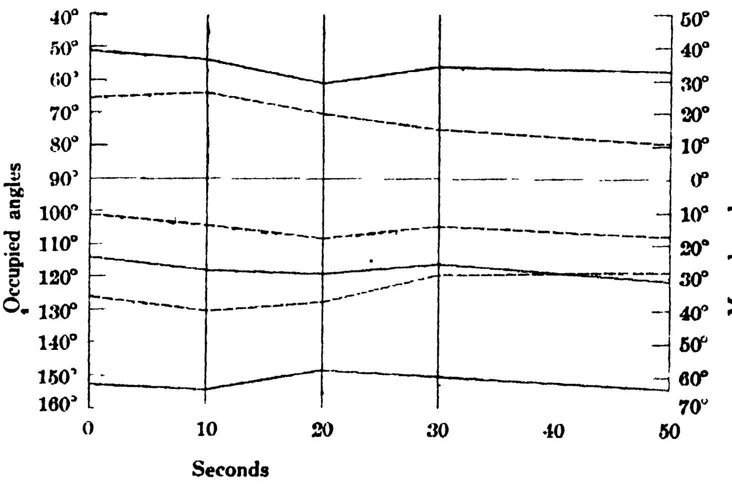


Fig. 10. $MgCl_2/8$

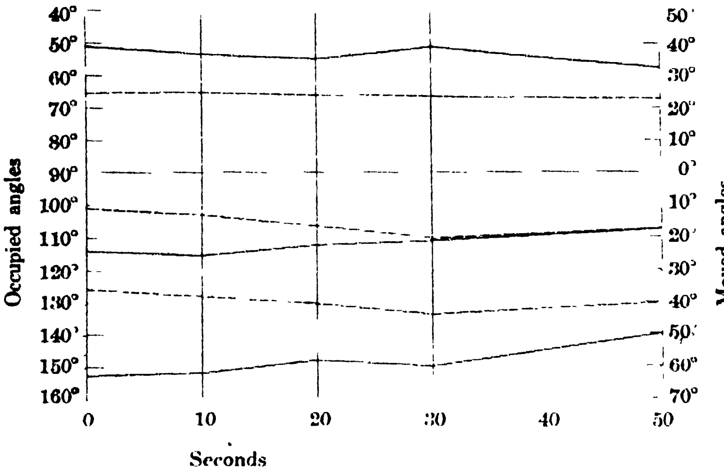


Fig. 11. $MgCl_2/4$

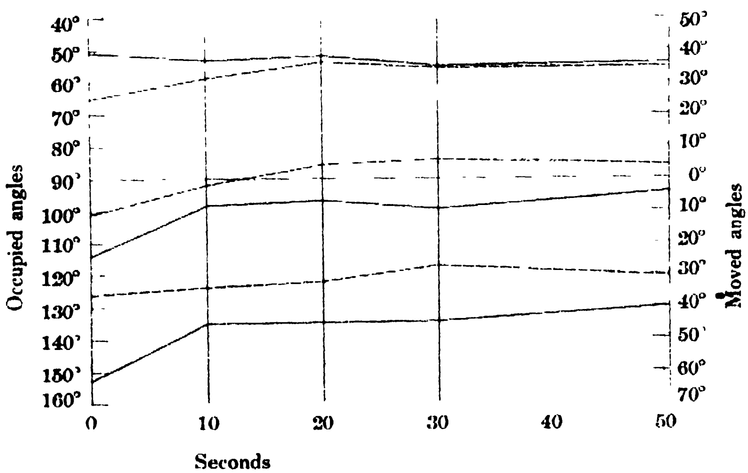


Fig. 12. $MgCl_2/2$

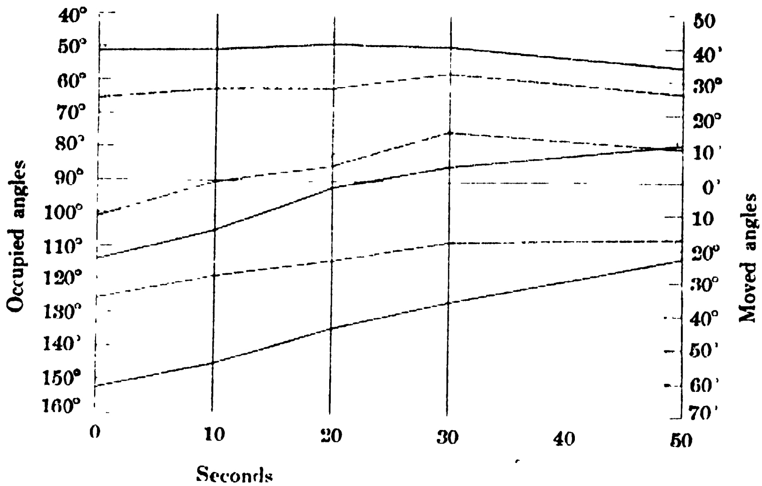


Fig. 13. $3\ MgCl_2/4$

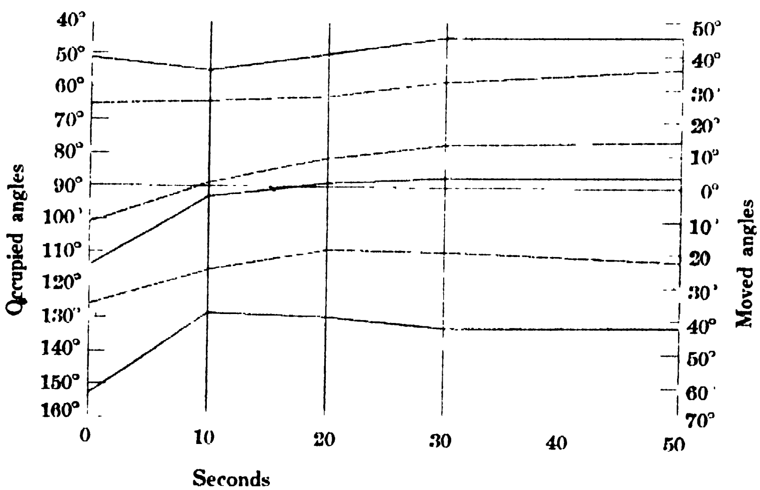
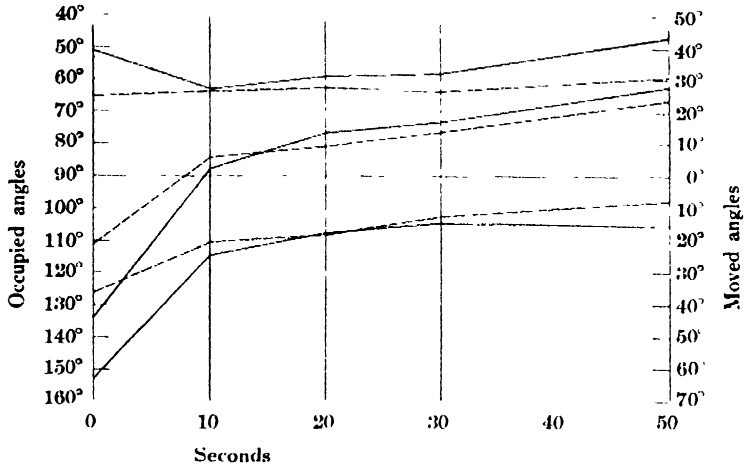


Fig. 14. $MgCl_2/1$



fourteen, therefore, it may be inferred that in the case of solutions of $MgCl_2$, within the limits of experiment, the negativity in the brain of the worms was weakened as the duration of immersion was prolonged.

D. Changes in Crawling.

From Tables 10 and 13 we may infer the following conclusions:

In the case of a stronger solution the strengthening of the backward functioning in crawling is caused by a relative weakening of the forward functioning in both the brain and the ventral nerve cord, while in the case of a weaker solution the weakening of the backward functioning may be caused mainly by a relative strengthening of the forward functioning in the ventral nerve cord.

III. EFFECTS OF WATER.

The experiments were carried out at a temperature of 20°C., on September 18 and 19, 1929, in order to determine the general tendency of the changes in the photic orientation of *Allolobophora foetida* in relation to raised concentrations of NaCl and $MgCl_2$, as stated in the last sections. In an experiment, the worms were immersed in drinking water for one of the varying periods of 0, 30, 60, 120, 180 or 300 seconds.

A. Movements of Unoperated Worms.

50 worms were tested individually for one of the definite periods of immersion.

Within the limits of experiment, not only did the worms manifest neither convulsions nor ejection of coelomic fluid, but the inactiveness which commonly occurred in the case of solutions of the inorganic salts was not detected.

Orientation. The changes in the magnitude of the occupied angles are shown in Table 15, and the frequency distribution of the worms is given in Table 16. From these tables, though there are some irregularities, we may infer that the changes in the magnitude are so insignificant that we cannot judge of the tendency of the degree of negative orientation either to increase or to decrease.

TABLE 15.

Duration of immersion in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0	80.82	101.66	110.84	79.94	110.46	120.52
30	80.12	100.08	109.96	79.36	106.42	117.06
60	81.32	104.62	113.30	80.46	113.46	123.00
120	80.74	97.04	106.30	78.28	108.30	120.02
180	77.96	100.92	112.96	83.20	113.88	120.68
300	80.46	100.69	110.23	81.31	112.83	121.52

TABLE 16.

Duration of immersion in seconds	5 cm. angles			10 cm. angles		
	0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
0	17	10	23	13	9	28
30	18	10	22	16	7	27
60	16	11	23	12	8	30
120	16	10	24	16	7	27
180	19	8	23	12	9	29
300	17	9	24	13	7	30

Crawling. According to Table 17, neither the returning nor winding movements occur, but the frequency of the backward crawling individuals may be increased with the prolongation of the duration of immersion.

TABLE 17.

Duration of immersion in seconds	5 cm. angles				10 cm. angles		Returning	Winding
	Forwards		Backwards					
	Directly	After posterior elongation	Directly	After anterior elongation	Forwards	Backwards		
0	50	0	0	0	50	0	0	0
30	50	0	0	0	49	1	0	0
60	50	0	0	0	50	0	0	0
120	50	0	0	0	50	0	0	0
180	49	0	1	0	49	1	0	0
300	50	0	0	0	50	0	0	0

B. Movements of Operated Worms.

25 worms were tested individually as an experiment.

Orientation. In Table 18, in which the changes in the magnitude of the occupied angles are shown, and in Table 19 in which the frequency distribution of the worms is given, as in the case of the worms not operated on, no notable tendency of the changes in the degree of positive orientation is detectable, even if a slight tendency to decrease in the degree may be shown with the increase in the number of seconds of submersion.

Crawling. Table 20 shows that in the case of the 10 cm. angles the number of backward crawling individuals is ultimately increased; nevertheless, at the outset (refer to the 5 cm. angles) the number of individuals which began to crawl backwards tended to decrease, while that of those which began to crawl forwards tended to increase.

TABLE 18.

Duration of immersion in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
0	63.00	69.96	96.96	50.16	59.08	98.92
30	59.48	65.08	95.60	51.40	60.04	98.64
60	60.48	66.52	96.04	50.76	58.20	97.44
120	59.52	65.32	95.80	53.12	59.92	96.80
180	59.92	67.76	97.84	54.[illegible]	65.76	101.60
300	63.96	72.28	98.32	55.32	64.68	99.36

TABLE 19.

Duration of immersion in seconds	5 cm. angles			10 cm. angles		
	0°-80°	81°-99°	100°-180°	0° 80°	81°-99°	100°-180°
0	16	4	5	16	4	5
30	15	7	3	17	3	5
60	18	3	4	18	3	4
120	17	5	3	19	3	3
180	16	5	4	18	2	5
300	13	7	5	18	2	5

TABLE 20.

Duration of immersion in seconds	5 cm. angles				10 cm. angles		Returning	Winding
	Forwards		Backwards					
	Directly	After posterior elongation	Directly	After anterior elongation	Forwards	Backwards		
0	16	1	8	0	17	8	0	0
30	15	0	8	2	15	10	0	0
60	15	0	7	3	15	10	0	0
120	14	0	9	2	14	11	0	0
180	14	0	7	4	13	12	0	0
300	15	0	5	5	15	10	0	0

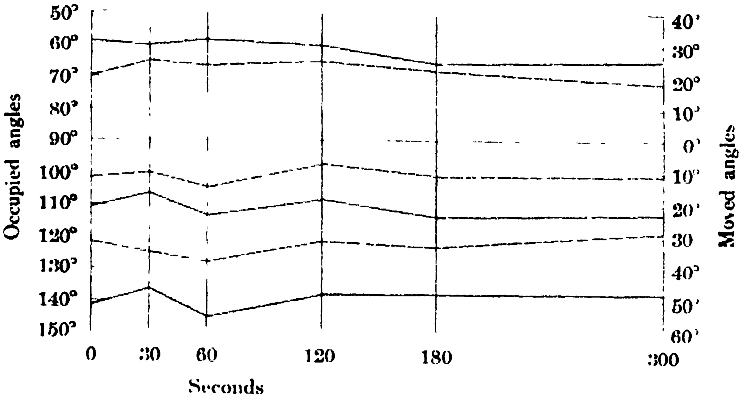
C. Changes of Negativity in the Brain.

Fig. 15 was plotted from the angular values of *P*, *A*, and *N* given together in Table 21. In this figure the broken lines denote the changes

TABLE 21.

Duration of immersion in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
	P	*A*	*N*	*P*	*A*	*N*
0	69.96	101.66	121.70	59.08	110.46	141.38
30	65.08	100.08	125.00	60.04	106.42	136.38
60	66.52	104.62	128.10	58.20	113.46	145.26
120	65.32	97.04	121.72	59.92	108.30	138.38
180	67.76	100.92	123.16	65.76	113.88	138.12
300	72.28	100.69	118.41	64.68	112.83	138.15

Fig. 15. Water



in the 5 cm. angles and the full lines those in the 10 cm. angles.

According to the figure, the value of N which denotesthe degree of negative orientation in the brain does not show any notable alteration, even if in the case of the 5 cm. angles the degree may tend to decrease with the prolongation of the duration of submersion.

D. Changes in Crawling.

From Tables 17 and 20 we may infer the following conclusions:

The strengthening of the backward movement in crawling, while in the water, was caused mainly by a relative strengthening of the backward functioning in the ventral nerve cord, but as the duration of immersion was prolonged the forward functioning of the crawling in the ventral nerve cord became stronger than the backward functioning.

Both the brain and the ventral nerve cord were not sufficiently affected by the water for the winding movement to be caused in the time-interval of the duration of the experiment.

IV. CHANGES OF ORIENTATION IN DIFFERENT CONCENTRATIONS OF NaCl.

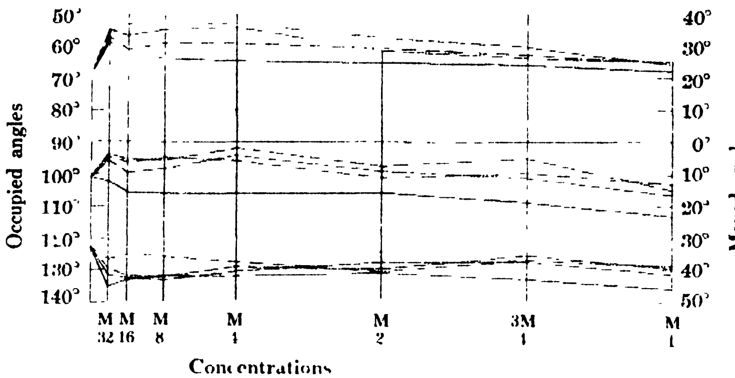
The object of the present investigation is to discover how the nature and degree of photic orientation would change with the rise in the degree of concentration, and to ascertain the character of the changes in the orientation in relation to the concentration of NaCl. Fig. 16 was prepared from Table 7, by a rearrangement of the data given in that table. The

data which were taken as the standards were the averages of the respective values of *P*, *A*, and *N* given in Table 21, viz. 67.82° for *P*, 100.84° for *A*, and 123.02° for *N* in the case of the 5 cm. angles, and 61.28° for *P*, 110.89° for *A*, and 139.61° for *N* in that of the 10 cm.

In Fig. 16, the changes in the 5 cm. and 10 cm. angles are shown

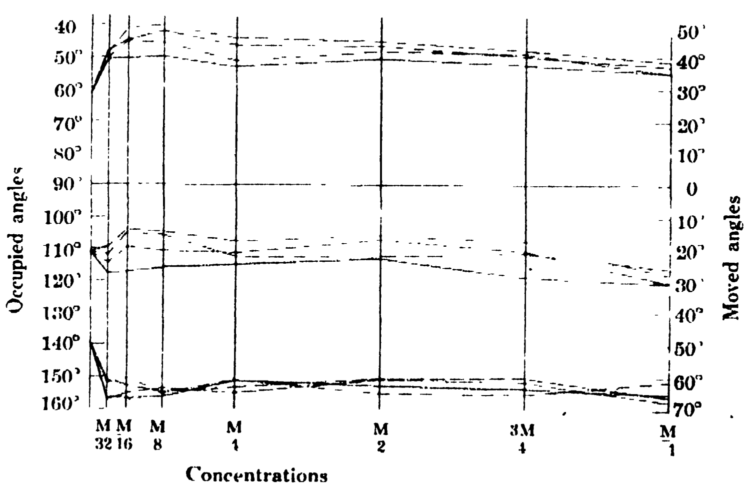
Fig. 16. NaCl

5 cm. angles



10 sec. 20 sec. 30 sec. 50 sec.

10 cm. angles



separately to avoid confusions. In the case of both angles, P or the degree of positive orientation shows an increasing tendency in a low concentration from 1/32 to 1/8, and then a decreasing tendency with the rise of concentration from 1/4 to 1/1; and N or the degree of negative orientation shows an increasing tendency with a rise from 1/32 to about 1/8, and then nearly constancy of degree, or a very slight increasing tendency, continuously with the rise of concentration. Consequently, A or the degree of negative orientation of the worms as a whole shows a slight increasing tendency with the rise of conentration to 1/1, and, if the increase in the degree follows the rise of concentration, this phenomenon may be caused mainly by a relative weakening of the degree of positive orientation in the ventral nerve cord in accordance with the statements[1)5)] already made.

V. CHANGES OF ORIENTATION IN DIFFERENT CONCENTRATIONS OF $MgCl_2$.

Fig. 17 was prepared from Table 14 by a rearrangement of the data given in that table, in order to see the character of the changes in orientation in relation to the concentration of $MgCl_2$. The data which were taken as the standards were the averages of the respective values of P, A, and N given in Table 21.

In the case of both the 5 cm. and 10 cm. angles, P or the degree of positive orientation shows a decreasing tendency in low concentrations, i. e. from 1/32 to 1/16 or 1/8, and then a constancy of the degree, or a very slight increasing tendency, with the rise of concentration from 1/4 to 1/1; and N or the degree of negative orientation shows an increasing tendency in the lower conentrations, i. e. from 1/32 to 1/8 or 1/4, and then a somewhat strongly decreasing tendency in the higher concentrations from 1/4 to 1/1. Thus, A or the degree of negative orientation of the worms as a whole is increased in the lower concentrations, from 1/32 to 1/8 or 1/4, and then is gradually weakened in the higher concentrations, from 1/4 to 1/1, and finally it alters to show the degree of positive orientation. If the weakening of the degree were to follow the rise of concentration, this phenomenon may be caused mainly by a relative weakening of the degree of negative orientation in the brain in accordance with the statements[2)6)] already given.

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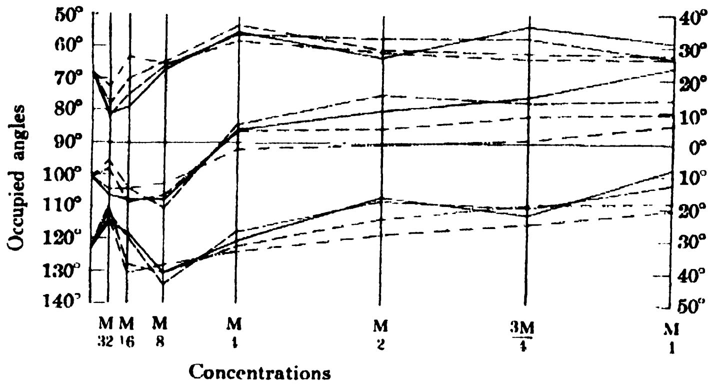
1) and 5) loc. cit.

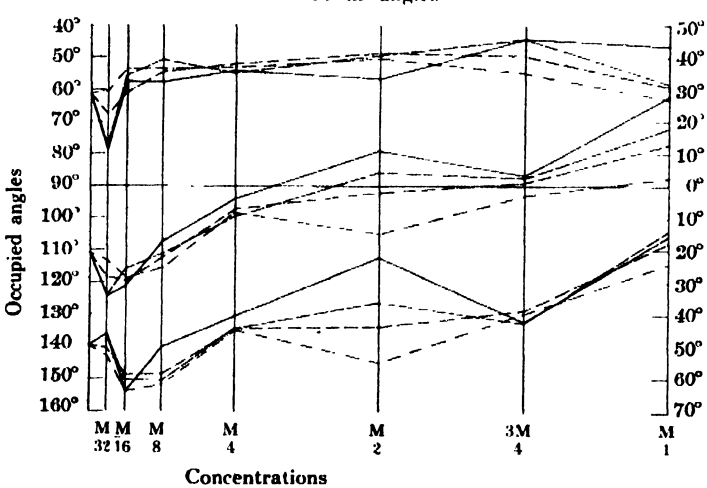
2) and 6) loc. cit.

Fig. 17. $MgCl_2$

5 cm. angles



10 cm angles



Salts tested:

$MgCl_2$	--	$CaCl_2$	$NaCl$	KCl
--	—	—	$NaBr$	KBr
--	--	—	NaI	KI
$MgSO_4$	$FeSO_4$	—	Na_2SO_4	K_2SO_4
$Mg(NO_3)_2$	—	$Ca(NO_3)_2$	$NaNO_3$	KNO_3

Changes in orientation in Allolobophora foetida:

$MgCl_2/2 + NaCl/1$, $CaCl_2/2 + KCl/1$; $K_2SO_4/1.7$; $NaNO_3/2$, $NaNO_3/1 + KNO_3/1$; $NaI/1.9$; $NaBr/1.9$, $NaBr/1.9 + KBr/1.9$; $MgCl_2/2 + MgSO_4/2$, $Na_2SO_4/4 + NaNO_3/2$, $Na_2SO_4/4 + NaCl/2$, $NaNO_3/2 + NaCl/2$ caused a weakening of the positively orientating functioning in the ventral nerve cord, and, at the same time, a weakening of the negatively orientating functioning in the brain.

$NaNO_3/1$ also caused a weakening of the positively orientating functioning in the ventral nerve cord, but a strengthening at first and then a weakening of the negatively orientating functioning in the brain.

$MgCl_2/2 + KCl/1$; $FeSO_4/1.7$; $Mg(NO_3)_2/2$, $Mg(NO_3)_2/2 + NaNO_3/1$; $NaI/1.9 + KI/1.9$; $Mg(NO_3)_2/2 + MgSO_4/2$ and $Mg(NO_3)_2/2 + MgCl_2/2$ caused a strengthening at first and then a weakening of the positively orientating functioning in the ventral nerve cord, and a weakening of the negatively orientating functioning in the brain.

$CaCl_2/2$, $KCl/1$, $MgCl_2/2 + CaCl_2/2$, $CaCl_2/2 + NaCl/1$; $MgSO_4/1.7$, $MgSO_4/2$, $Na_2SO_4/1.7$, $Na_2SO_4/4$, $MgSO_4/1.7 + FeSO_4/1.7$, $MgSO_4/1.7 + Na_2SO_4/1.7$, $FeSO_4/1.7 + Na_2SO_4/1.7$, $MgSO_4/1.7 + K_2SO_4/1.7$, $FeSO_4/1.7 + K_2SO_4/1.7$, $Na_2SO_4/1.7 + K_2SO_4/1.7$; $Ca(NO_3)_2/2$, $KNO_3/1$, $Mg(NO_3)_2/2 + Ca(NO_3)_2/2$, $Ca(NO_3)_2/2 + NaNO_3/1$, $Mg(NO_3)_2/2 + KNO_3/1$, $Ca(NO_3)_2/2 + KNO_3/1$; $KI/1.9$; and $KBr/1.9$ caused a strengthening at first and then a weakening of the positively orientating functioning in the ventral nerve cord, and also a strengthening at first and then a weakening of the negatively orientating functioning in the brain.

When the worms were immersed in the single or mixed salt solutions, they showed or ought to show, invariably, after a long run of time, either the random movement while live or no movement when dead (in both cases the average occupied angle must be 90°) as a definitive effect of such strong solutions, even though most of the solutions caused a streng-

thening of either or both of the positively orientating functioning in the ventral nerve cord and the negatively orientating functioning in the brain at the beginning of immersion.

Besides those above-mentioned, special tendencies of change in orientation different from them were shown by NaCl and $MgCl_2$:

NaCl. This salt disclosed rather diverse effects on the photic orientation in relation to concentration, temperature and month. In the experiments carried on at a temperature of 19.5°–20.5°C., during June 10–22, 1926, we found in a normal solution a tendency to cause a weakening of the positively orientating functioning in the ventral nerve cord and a strengthening of the negatively orientating functioning in the brain, and even the effect of NaCl+KCl was to manifest a general tendency of this sort, though it caused a strengthening of the positively and a weakening of the negatively orientating functioning at the beginning of immersion. In the experiments carried on at a temperature of 25°C., during August 17–23, 1928, we were able to obtain, even in the case of a 1/2 normal solution, nearly the same results. In the experiments, however, which were carried on at a temperature 26.1°–26.7°C., during July 15–27, 1929, in spite of the fact that all the solutions, from a 1/32 to a normal, showed a tendency to strengthen the negatively orientating functioning in the brain, in the ventral nerve cord a tendency to weaken the positively orientating functioning was detectable only in a stronger concentration, while in a weaker concentration it tended to cause a strengthening at first and then a weakening of the positively orientating functioning.

$MgCl_2$. This salt also exhibited somewhat diverse effects on the photic orientation in Allolobophora in relation to concentration, temperature and month. In the experiments carried on at a temperature of 19.5–20.5°C., during June 10–22, 1926, with a 1/2 normal solution there was evidently a tendency to cause a strengthening of the positively orientating functioning in the ventral nerve cord and a weakening of the negatively orientating functioning in the brain. In the experiments carried on at a temperature of 23°–25°C., during August 17–23, 1928, we were able to obtain also with a 1/2 normal solution nearly the same results, even though this solution tended to cause a slight weakening of the positively orientating functioning in the ventral nerve cord. In the experiments, however, which were carried on at a temperature of 26.7°–27.8°C., during July 28–August 10, 1929, in spite of the fact that all the solutions, from a 1/32 to a normal, showed a tendency to weaken the negatively orientating functioning in the brain, a tendency to strengthen the positively orientating functioning

in the ventral nerve cord was detectable only in the case of a higher concentration, i.e. above 1/2, and in a lower concentration it tended to cause a strengthening at first and then a weakening of positively orientating functioning, even a tendency to weaken continuously having been shown in the case of a 1/32 normal solution.

Probable effective order of the cations in causing changes of orientation in Allolobophora foetida. (This part of the inferences appears even to us very doubtful.)

Chlorides —	
in the brain	Ca > Mg > Na or K
in the ventral cord	Ca > Na or K Mg
Sulphates —	
in the brain	Na or K > Mg Fe
in the ventral cord	Mg > Fe Na or K
Nitrates —	
in the brain	K Ca Mg Na
in the ventral cord	Mg Ca > Na K
Iodides —	
in the brain	Na > K
in the ventral cord	K > Na
Bromides —	
in both the brain and the ventral cord	Na > K

Changes in backward crawling in Allolobophora foetida:

$CaCl_2/2$, $MgCl_2/2+CaCl_2/2$, $CaCl_2/2+NaCl/1$; $Mg(NO_3)_2/2+Ca(NO_3)_2/2$, and $Ca(NO_3)_2/2+KNO_3/1$ caused backward crawling mainly by a relative weakening of the forward functioning in the brain.

$KCl/1$, $NaCl/1+KCl/1$; $MgSO_4/1.7$, $Na_2SO_4/1.7$, $Na_2SO_4/1$, $MgSO_4/1.7+Na_2SO_4/1.7$, $MgSO_4/1.7+K_2SO_4/1.7$; $NaNO_3/1$, $NaNO_3/2$, $KNO_3/1$; $NaI/1.9$, $KI/1.9$, $NaI/1.9+KI/1.9$; $NaBr/1.9$, $KBr/1.9$, $NaBr/1.9+KBr/1.9$; $Na_2SO_4/4+NaNO_3/2$, $Na_2SO_4/4+NaCl/2$, and $NaNO_3/2+NaCl/2$ caused backward crawling mainly by a relative weakening of the forward functioning in the ventral nerve cord.

$MgCl_2/2+NaCl/1$, $MgCl_2/2+KCl/1$, $CaCl_2/2+KCl/1$; $MgSO_4/2$, $FeSO_4/1.7$, $K_2SO_4/1.7$, $MgSO_4/1.7+FeSO_4/1.7$, $FeSO_4/1.7+Na_2SO_4/1.7$, $FeSO_4/1.7+K_2SO_4/1.7$, $Na_2SO_4/1.7+K_2SO_4/1.7$; $Mg(NO_3)_2/2$, $Ca(NO_3)_2/2$, $Mg(NO_3)_2/2+NaNO_3/1$, $Ca(NO_3)_2/2+NaNO_3/1$, $Mg(NO_3)_2/2+KNO_3/1$, $NaNO_3/1+KNO_3/1$; $MgSO_4/2+Mg(NO_3)_2/2$, $MgSO_4/2+MgCl_2/2$, and $Mg(NO_3)_2/2+MgCl_2/2$ caused backward crawling by a relative weakening

of the forward functioning in both the brain and the ventral nerve cord.

In a solution of NaCl weaker than 1/2 normal a strengthening of the backward functioning in crawling was caused by a relative weakening of the forward functioning in both the brain and the ventral nerve cord, while in a solution stronger than 3/4 normal it was caused by a relative weakening of the forward functioning in the ventral nerve cord.

In a solution of $MgCl_2$ stronger than 3/4 normal a strengthening of the backward functioning was caused by a relative weakening of the forward functioning in both the brain and the ventral nerve cord, while in a solution weaker than 1/2 normal a weakening of the backward functioning might be caused mainly by a relative strengthening of the forward functioning in the ventral nerve cord.

In water a strengthening of the backward crawling movement was caused mainly by a relative strengthening of the backward functioning in the ventral nerve cord, but as the duration of immersion was prolonged the forward functioning in the ventral nerve cord became stronger than the backward functioning.

Probable effective order of the cations in causing backward crawling in Allolobophora foetida:

Chlorides —		Ca $\geqq$ Mg $\geqq$ K or Na
Sulphates —		Fe > Mg > K > Na
Nitrates —	indeterminable, owing to the discordant results from the experiments, but	? Mg or Ca $\geqq$ Na or K
Iodides —		indeterminable
Bromides —		indeterminable

General conception of the mechanism causing changes in the tropism reaction of animals. With regard to the phototactic movements in the nauplii of *Balanus perforatus*, EWALD[7] gives the following statements: "Isotonic sodium chloride solution, pure or added to natural seawater, makes negative animals positive and positive animals more positive. If sufficiently in excess of the other salts, it inhibits negative reaction entirely. Isotonic potassium chloride solution, added to natural seawater, acts in the same direction, though less effectively. Isotonic calcium chloride solution, added to natural seawater, makes the larvae lose their power of reaction to light stimuli, causing them to swim about at random without negative or positive orientation. Magnesium chloride or sulphate solution acts as

7) EWALD, W. F. 1912. On artificial modification of light reaction and the influence of electrolytes on phototaxis. Journ. Exp. Zoöl., Vol. 13, Pp. 591-612.

an antagonist to sodium. Added in the proportion prevailing in natural seawater to pure NaCl solution, $MgCl_2$ brings about the negative reaction which is suspended in pure NaCl solution. There is no difference in response to photic stimuli between larvae in the sodium magnesium mixture and inpure seawater. For a normal production of light reaction it is necessary to have the correct production of sodium on one side and magnesium on the other. Hypertonic solutions of NaCl or $MgCl_2$ had a strong positivating effect, hypotonic solution of NaCl had an equally obvious negativating effect." As to phototactic reactions in *Hyalella*, JACKSON[8] states that "some potassium salts made them weakly positive; potassium bromide and potassium chlorate produced no marked change in their phototactic response, nor did any of the sodium salts, or magnesium sulphate." And the following statements are given by KANDA[9] in relation to *Arenicola* larvae: "In seawater made hypertonic by addition of NaCl or KCl some larvae (20–35 per cent.) become negative. In seawater made hypertonic with $CaCl_2$, $MgCl_2$ or $MgSO_4$, no reverse was observed. Hypertonic sodium and potassium chloride solutions added to the artificial seawater reversed the positive heliotropism of the majority of the larvae."

As to the other fields of tropism reaction two following statements may be referred:

"Any chemical in the concentration used," states ALLEE[10] with regard to rheotaxis in *Asellus*, "will cause a decrease in the positive rheotactic reaction, but the chlorine salts of calcium and strontium cause this decrease usually without a preliminary stimulation. Magnesium chloride while in the main similar in action often causes preliminary stimulation and barium chloride is still more stimulating, resembling the alkali metals in its effect. In the cations such as potassium which are highly stimulating the depression is a toxic effect while in depressing cations as calcium, rheotaxis is depressed long before toxicity symptoms appear. There is a marked antagonism between the effect of potassium and calcium chlorides and a less marked one between the chlorides of sodium and magnesium."

"According to COEHN and BARRAT," states BANCROFT[11] in retation to

[8] JACKSON, H. H. T. 1910. The control of phototactic reactions in *Hyalella* by chemicals. Journ. Comp. Neur., Vol. 2, Pp. 259–263.

[9] KANDA, S. 1919. On the reversibility of the heliotropism of *Arenicola* larvae by chemicals. Biol. Bull., Vol. 36, Pp. 149–166.

[10] ALLEE, W. C. 1916. Chemical control of rheotaxis in *Asellus*. Journ. Exp Zoöl., Vol. 21, Pp. 163–198.

[11] BANCROFT, F. W. 1906. The control of galvanotropism in *Paramecium* by chemical substances. Univ. Calif. Publ., Physiol., Vol 3, No. 4, Pp. 21–31.

the galvanotaxis in *Paramecium*, "the backward swimming to the anode must depend only upon the osmotic pressure, and not upon the chemical nature of the solution. It has been found, however, that as regards this anodal swimming paramecia behave very differently to equimolecular salt solutions having chemical differences."

From the above it may be understood that the action of chemicals differs according not only to their different properties[12] and to their different concentrations, but even relatively to different species of animals. Besides these relations, differences in temperature and season cause an alteration in the photic orientation[13][14]. Moreover, polychaete larvae and very young earthworms show positive orientation, while their adults show negative orientation towards the same light source[15]; fatigued specimens of Allolobophora show positive orientation, while fresh ones show negative orientation[16] and not only do axially different parts of the body of Allolobophora show different reactions[16], but even a single specimen can alter its orientation while it is crawling forwards or backwards (trial and error). Thus it may be definitely stated that even within the limits of the Allolobophora, the animal reacts differently to light or to any stimulating source according to the difference not only in its environmental conditions externally, but in its own physiological conditions, internally.

NOMURA[16] believes that phototaxis in *Allolobophora foetida* must originally depend directly on the functioning of the entire central nervous system, because this phenomenon is exhibited as a bodily movement, even if many indirect factors such as changes in the osmotic pressure, in the metabolic state, in receptors and in the nerve endings, etc. may be enumerated, and in 1926 he concluded that the orientation of Allolobophora is determined by the antagonistic functioning of the brain and of the ventral nerve cord, orientation being distinct from phototaxis, which is

[12] NOMURA, E. 1926. Effect of chemicals on phototaxis in *Allolobophora foetida* (SAV.), and its analysis based upon the antagonistic relation between the functionings of the brain and of the ventral nerve cord. Sci. Rep. Tôhoku Imp. Univ., 4th ser., Vol. 2, Pp. 1-51.

[13] NOMURA, E. and OHFUCHI, S. 1926. Effect of heat on phototaxis in *Allolobophora foetida* (SAV.), and its analysis based upon the antagonistic relation between the functionings of the brain and of the ventral nerve cord *Ibid.*, Vol. 2, Pp. 105-126.

[14] NOMURA. E. and OHFUCHI, S. 1928. Seasonal changes of photic orientation in *Allolobophora foetida. Ibid.*, Vol. 3, Pp. 97-112.

[15] NOMURA, E. 1928. Kwankei-Dôbutsu no Jyôriku to Tekiô (Landing and adaptation of Annelids). Nippon-Gakujutsu-Kyôkwai-Hôkoku, Vol. 4, Pp. 470-477.

[16] NOMURA, E. 1926. Effect of light on the movement of the earthworm, *Allolobophora foetida* (SAV). Sci Rep. Tôhoku Imp. Univ., 4th ser., Vol. 1, Pp. 293-409.

caused by forward or backward crawling together with positive or negative orientation.

Now, we wish to state as a general conception that the apparent orientation is determined definitively by the antagonism between the degrees of positive and negative orientations occurring in the entire central nervous system, and that, therefore, the apparent orientation is changeable at any time when the antagonistic relation changes. Thus tropism reactions ought to be determined by the orientation in combination with the forward or backward crawling, which is also determined by the antagonism between the functionings in the central nervous system.

Finally, on the basis of the above conception, we wish to maintain that all the factors which alter the antagonistic relations alter also the tropism reaction[17]. July 1, 1932.

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[17] NOMURA, E. 1930. Kôsei no Henkwa ni tsuite (On changes in orientation Nippon-Gakujutsu-Kyôkwai-Hôkoku, Vol. 6. Pp. 466–173.

ON THE DAILY FLUCTUATION OF THE OSMOTIC VALUE IN PLANTS, II.[1]

By

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(Received August 1, 1932)

The regular diurnal fluctuation of the osmotic value in the normal state of the cell in leaves is well known by the works of a number of investigators, such as URSPRUNG and BLUM (1916), KORSTIAN (1924), WALTER (1929, 1931), BRAUN-BLANQUET and WALTER (1931), and VOLK (1931)[2], and it has been dealt with generally as an indication of the diurnal change in the water balance; while BLAGOWESTSCHENSKI (1928) proved by means of the plasmolytic method a similar fluctuation in the osmotic value at incipient plasmolysis in various plants. The writer (1931) observed by the plasmolytic method that the osmotic value underwent a regular diurnal fluctuation in some plants on Mt. Hakkôda, although it was not found in a large number of plants there.

The plasmolytic method differs substantially from the cryoscopic in giving not the osmotic value in the normal state but the value at incipient plasmolysis — in other words, when the cell wall is not at all distended and the cell sap has practically a certain definite volume. Accordingly, in the determination of the fluctuation of the osmotic value by means of the plasmolytic method in the same tissue in a relatively short period of time, we can deal with the quantitative change of osmotically effective substances in cell sap, apart from the status of the water balance which can be estimated only by determining the water content. By the cryoscopic method, on the contrary, we can know the osmotic value in the normal state of the cell which is the result of both the water balance and the amount of osmotically effective substances in cell sap.

The fact that the osmotic value at incipient plasmolysis shows a diurnal fluctuation is to be regarded as the only result of fluctuation of the amount of osmotically effective substances in cell sap, which seems to be induced by the water deficit occurring in the course of daytime.

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[1] Contributions from the Mt. Hakkôda Botanical Laboratory. No. 16.

[2] Most of those authors made use of the cryoscopic method, while URSPRUNG and BLUM used the plasmolytic method correcting the results for the normal state of the cell by taking into consideration the change of the volume in plasmolysis.

The present investigation was conducted to ascertain these relations, using as material a species of plant, *Polygonum sachalinense*, in which a remarkable diurnal fluctuation of the osmotic value at incipient plasmolysis was proved in the writer's previous work. The osmotic value at incipient plasmolysis and the water content together with the amounts of sugars and starch of the leaves were compared in the materials taken before sunrise (at about 4 a.m.) and in the afternoon (at about 3 p.m.). The investigation was carried out for twelve days in July and August of 1931. The osmotic value was determined in the epidermal cells of the upper side of the leaf, while the water content, sugars and starch were all estimated in the whole tissues of the leaf blade (except the midrib).

MATERIAL AND METHODS

Two individuals of *Polygonum sachalinense* growing on open ground in the neighbourhood of the Mt. Hakkôda Botanical Laboratory were used. This perennial herb has very large soft leaves, the height reaching about three meters. In earlier July when a part of the experiments was made they were in active growth and about two meters high; whereas in the middle of August when the rest of the experiments were set about they attained full growth and were blooming.

The osmotic value was determined by the plasmolytic method with sucrose solutions with a difference of 0.02 mol., otherwise in the same way as in the previous work.

The determination of sugars and starch was carried out as follows: Fresh leaves were killed by inserting into alcohol, and finely powdered after drying. The powdered material was extracted with water over night in an incubator at 37 in the presence of toluene. The extract, together with the alcohol used, was tested for simple sugars and sucrose. The inversion was made by boiling with hydrochloric acid. The residue was boiled with water, and then kept over night at 37 under the addition of Taka-diastase and toluene. After filtration, the filtrate was further hydrolyzed with the acid, and glucose thus produced was determined, on which the original amount of starch was calculated. Simple sugars were determined by means of the WOOD-OST copper carbonate method [COLE (1928) p. 177].

In the determination of the osmotic value and the analysis, mature leaves from about the same height on the shoots were taken as material. While in the 1st series of experiments different leaves were taken for

the morning and afternoon tests on each day, the same leaf was used for the tests in the other series, the half including the midrib being left intact for the afternoon test.

Sugars and starch, as well as the water content in the 1st and 3rd series of experiments, were always estimated in all the leaves used for the osmotic value determination.

RESULTS

Results obtained are shown in the following tables: The percentages are all on dry weight basis. The fluctuation is indicated by the difference between the morning and afternoon values in the percentage to the former, a decrease being marked with a negative sign.

Evaporation (Ev) was determined by the LIVINGSTON spherical atmometer, and duration of sunshine (Ss) by the JORDAN sunshine recorder. Evaporation is indicated in cc.—that during the daytime (7 a.m. to 7 p.m.) and that during the preceding night (7 p.m. to 7 a.m.) are shown separately, the latter in brackets. Duration of sunshine is in hr.—that during the time from sunrise to 3 p.m. is shown. Temperature (T), in C., is represented by those at 7 a.m. and 1 p.m., moreover that at 9 p.m. in the preceding evening being shown in brackets.

1st series of experiments—with "individual no. 1".

July 5: Rainy
July 6: Fair
July 7: Fair—Ev = 14.9(0.7), Ss = 5.5, T = 13–16(9)
July 8: Fair—Ev = 14.9(1.2), Ss = 6.3, T = 12–17(10)

			Morning	Afternoon	Fluctuation %
July 9: Cloudy, then fair (windy) Ev = 13.0(0.6) Ss = 2.0 T = 12–13(10)	Osmotic value *mol. sucrose*	Shoot no. 1–6*	0.30–0.42	0.42–0.46	
		Mean	**0.347**	**0.427**	**26**
	Water content %		268	221	–18
	Simple sugars %		1.10	1.25	14
	Sucrose %		2.10	2.63	25
	Starch %		2.61	5.90	126

*One leaf was taken from each shoot. Only in this series of experiments different leaves were used in the morning and the afternoon respectively.

			Morning	Afternoon	Fluctuation %
July 10: Fair (windy) Ev = 14.8(0.6) Ss = 8.0 T = 9-13(8)	Osmotic value	Shoot no. 1-6	0.34-0.40	0.38 0.46	
		Mean	**0.367**	**0.413**	**13**
	Water content		279	229	-18
	Simple sugars		1.10	1.09	- 1
	Sucrose		2.23	1.91	-14
	Starch		5.15	8.19	59
July 11: Foggy Ev = 0(0.2) Ss = 0.2 T = 8-8(7)	Osmotic value	Shoot no. 7-12	0.34 0.40	0.40-0.46	
		Mean	**0.360**	**0.423**	**18**
	Water content		258	245	- 5
	Simple sugars		1.33	1.66	25
	Sucrose		2.44	3.62	48
	Starch		5.08	11.31	124
July 12: Foggy Ev = 0(0) Ss = 0 T = 8-8(6)	Osmotic value	Shoot no. 7-12	0.34-0.40	0.32-0.42	
		Mean	**0.367**	**0.367**	**0**
	Water content		258	253	- 2
	Simple sugars		2.19	1.77	-19
	Sucrose		2.40	2.76	15
	Starch		7.24	8.80	22

2nd series of experiments — with " individual no. 2 ".

August 10: Rainy
August 11: Fair
August 12: Cloudy, occasionally cleared — Ev = 9.0(0.7), Ss = 0.8, T = 15–19(15)
August 13: Fair, frequently clouded Ev = 11.4(1.0), Ss = 4.0, T = 17–20(15)

				Morning	Afternoon	Fluctuation %
August 14: Fair, frequently clouded	Osmotic value *mol. sucrose*	Shoot no. 1	Leaf no. 1	0.38	0.48	26
			,, no. 2	0.40	0.50	25
		,, no. 2	,, no. 1	0.38	0.46	21
		Mean		**0.387**	**0.480**	**24**

Ev = 13.4 (2.0) Ss = 5.0 T = 19–21 (15)	Water content %	Shoot no. 1	Leaf no. 1	219	180	–18
			,, no. 2	209	181	–13
		,, no. 2	,, no. 1	210	182	–13
		Mean		**213**	**181**	**–15**
August 15: Fair	Osmotic value	Shoot no. 1	Leaf no. 3	0.40	0.44	10
			,, no. 4	0.40	0.50	25
		,, no. 2	,, no. 2	0.44	0.44	0
		Mean		**0.413**	**0.460**	**10**
Ev = 20.6 (6.2) Ss = 8.2 T = 21–25 (18)	Water content	Shoot no. 1	Leaf no. 3	204	185	9
			,, no. 4	212	184	–13
		,, no. 2	,, no. 2	214	200	7
		Mean		**210**	**190**	**–10**
August 16: Fair	Osmotic value	Shoot no. 3	Leaf no. 1	0.42	0.46	10
			,, no. 2	0.42	0.42	0
		,, no. 4	,, no. 1	0.40	0.44	10
		Mean		**0.413**	**0.440**	**7**
Ev = 17.4 (5.8) Ss = 7.0 T = 24–27 (20)	Water content	Shoot no. 3	Leaf no. 1	232	213	– 8
			,, no. 2	240	228	– 5
		,, no. 4	,, no. 1	234	216	– 8
		Mean		**235**	**219**	**– 7**
August 17: Cloudy, frequently cleared — Ev 11.6 (5.0), Ss = 0.2, T = 20–27 (19)						
August 18: Fair	Osmotic value	Shoot no. 3	Leaf no. 3	0.40	0.46	15
			,, no. 4	0.36	0.44	22
		,, no. 4	,, no. 2	0.40	0.48	20
		Mean		**0.387**	**0.460**	**19**
Ev = 22.1 (4.3) Ss = 5.6 T = 21–26 (20)	Water content	Shoot no. 3	Leaf no. 3	234	195	–17
			,, no. 4	238	194	– 18
		,, no 4	,, no 2	238	206	–13
		Mean		**237**	**198**	**16**

3rd series of experiments — with "individual no. 1".

				Morning	Afternoon	Fluctuation %
August 19:	Osmotic value mol. sucrose	Shoot no 1	Leaf no. 1	0.42	0.50	19
Fair, then cloudy		,, no. 2	,, no. 1	0.42	0.48	14
Ev- 16.6(3.4)		,, no. 3	,, no 1	0.40	0.46	15
Ss - 6.0		,, no. 4	,, no. 1	0.38	0.44	16
T 24-26(19)		,, no. 5	,, no. 1	0.42	0.48	14
		,, no. 6	,, no. 1	0.42	0.50	19
		Mean		**0.410**	**0.477**	**16**
	Water content %			187	165	-12
	Simple sugars %			1.23	1.56	27
	Sucrose %			2.64	3.10	17
	Starch %			3.35	7.45	122
August 20:	Osmotic value	Shoot no 1	Leaf no. 2	0.42	0.50	19
Fair		.. no 2	,, no. 2	0.42	0.52	24
Ev - 24.3(5.4)		,, no. 3	,, no 2	0.38	0.46	21
Ss - 8.0		,, no. 4	,, no. 2	0.40	0.48	20
T -21-25(22)		,, no. 5	,, no. 2	0.40	0.50	25
		,, no. 6	,, no. 2	0.40	0.50	25
		Mean		**0.403**	**0.493**	**21**
	Water content			182	160	-12
	Simple sugars			1.64	1.38	-16
	Sucrose			2.97	4.31	45
	Starch			2.74	5.35	95

August 21: Fair, frequently clouded — Ev-17.8(9.0), Ss=1.7, T-21-23(21)
August 22: Fair or cloudy in the forenoon, rainy (3 n.m.) in the afternoon - Ev-8.5(3.0), Ss -2.6, T=23-23(20)

August 23: Rainy (0.5 mm.) in the preceding night; cloudy, then heavy rain (ca. 50 mm.) in the afternoon; Ev = 9.2(3.4); Ss = 1.2; T = 21–22(19)	Osmotic value	Shoot no. 1	Leaf no. 3	0.44	0.48	9
		,, no. 2	,, no. 3	0.40	0.48	20
		,, no 3	,, no. 3	0.36	0.40	11
		,, no. 4	,, no. 3	0.38	0.48	26
		,, no. 5	,, no. 3	0.42	0.46	10
		,, no. 6	,, no. 3	0.42	0.46	10
		Mean		**0.403**	**0.460**	**14**
	Water content			189	171	10
	Simple sugars			2.17	1.79	−18
	Sucrose			2.57	3.39	32
	Starch			3.29	5.15	57
August 24: Cloudy; Ev = 5.0(0); Ss = 0; T = 13–15(17)	Osmotic value	Shoot no. 1	Leaf no. 4	0.40	0.48	20
		,, no. 2	,, no. 4	0.40	0.50	25
		,, no. 3	,, no 4	0.38	0.48	26
		,, no. 4	,, no. 4	0.40	0.44	10
		,, no. 5	,, no. 4	0.40	0.46	15
		,, no. 6	,, no. 4	0.40	0.46	15
		Mean		**0.397**	**0.470**	**18**
	Water content			193	170	12
	Simple sugars			1.99	2.16	9
	Sucrose			2.26	3.68	63
	Starch			3.84	3.95	3

DISCUSSION AND CONCLUSIONS

In the 1st series of experiments we can compare dry days with sunshine (the 9th and 10th) and humid days almost without sunshine and with the air saturated with vapour (the 11th and 12th). The osmotic value was higher in the afternoon than in the morning, except the 12th on which it remained the same throughout, but sank again to an almost definite value every morning. The water content always decreased in the afternoon, though the decrease was very little on humid days. It is clear that the

water deficit brought about in the daytime was recovered in the night, since the water content showed a rather constant value every morning.

During the 2nd series of experiments the climatic conditions were arid, fine warm days with strong evaporation and full sunshine continuing. The increase of the osmotic value during the daytime was observed in almost every leaf, while the morning value showed no remarkable difference. The water content always showed a decrease in the afternoon, but the water balance was recovered in the night. It is noteworthy that as a rule the larger the increase in the osmotic value the larger was the decrease in the water content in individual leaves — this fact could be proved to some extent also in the other series.

In the 3rd series of experiments, the first two days were dry, whereas the subsequent two days were more or less humid. The increase of the osmotic value and the decrease of the water content were pronounced every day. The osmotic value and the water content remained almost constant in the morning on either dry or humid days.

The above mentioned results can be summarized as follows:

1) As a rule, the osmotic value at incipient plasmolysis was higher (up to nearly 30%) and the water content smaller in the afternoon than in early morning. This fact shows that the amount of osmotically effective substances in cell sap increases during the daytime, water deficit being brought about at the same time. The probable diurnal fluctuation of the osmotic value in the normal state of the cell may be not only due to the change in the water balance but to the new formation of osmotically effective substances.

2) Moreover, a parallelism in the rate of increase of the osmotic value and the rate of decrease of the water content was clearly recognized — in other words, the larger was the water deficit, the more osmotically effective substances were formed.

3) The osmotic value and the water content remained rather constant every morning regardless of the daily climatic conditions. Namely, the water deficit brought about in the daytime could be fully recovered in the night and, on the other hand, the amount of osmotically effective substances sank to a more or less definite value in the morning. It is to be noted that the weather was rather changeable on this mountain and the environmental conditions were different from those of the experiments of VOLK (1931) and PISEK and CARTELLIERI (1932), who observed relatively high morning values in the osmotic value in the normal state of the cell during a dry period. Throughout the period of the writer's experiments the water

content of the soil around the roots of the plants in question varied within the range from 23 to 36%.

Besides, we can find still other remarkable facts that the osmotic value was higher in August than in July in the same individual — even in the same shoots — and that, on the other hand, the water content was smaller in August that in July — the mean osmotic value in the morning was 0.36 mol. in July and 0.40 mol. in August, while the mean value of the water content was 266% in July and 188% in August. Such distinct seasonal differences might be due to internal causes in the plant, which was in active growth in July while in August the growth has been practically completed and it was blooming. The fact that the osmotic value increases and the water content decreases in the course of the growing period, agrees with what PISEK and CARTELLIERI (1932) noticed in various plants. So far as the osmotic value is concerned, the same thing was observed by FIRBAS (1931) in moor plants. BLAGOWESTSCHENSKI (1928) also observed that the osmotic value reached the maximum at the blooming time in a cotton plant.

The real nature of osmotically effective substances in the cell sap has not yet been thoroughly ascertained, but generally sugars have been supposed to be the most important ones among those substances [LEWIS and TUTTLE (1920), LODE (1924), etc.], although salts are known to play a great rôle in plants growing on saline soil or in saline water [FITTING (1911), FABER (1913, 1923), BRAUN-BLANQUET and WALTER (1931, p. 728 ff.)]. On the other hand it is known from the researches of HORN (1923) and AHRNS (1924) that a loss of water from the cell — such as in wilting — induces an increase in the sugar content at the cost of starch. ILJIN (1929) not only observed that even a slight loss of water causes an increase in the sugar content, but ascertained that the same species of plant shows a higher sugar content of cell sap when grown in a dry habitat than in a moist one, and that ecological groups of plants which are known to be characterized with low osmotic values — such as succulents — contain smaller amounts of sugars. W. MÜLLER[1] pointed out, on the basis of data obtained by RANCKEN and BENDER, higher osmotic values in the groups of mosses poor in starch (presumably rich in sugar instead) than in those rich in starch.

PISEK and CARTELLIERI (1932) ascertained in species of plants including both sun and shade plants that the osmotic value determined by the

[1] Cf. *Zeitschr. f. Botan.*, 11 (1919), 231.

cryoscopic method rises in the course of daytime while the water content sinks, and they suggested the possibility of participation of sugars, which are considered to be transformed from starch in connection with water deficit. WALTER (1931) was of a similar opinion as those authors who recognized the rôle of sugars, for he pointed out the inverse relation between the diurnal curves of the water content and the amount of sugars, especially disaccharides, on the basis of results obtained by KOKIN[1] while MAXIMOW[2] attributed the diurnal fluctuation of the osmotic value in the normal state of the cell to the change of the water balance exclusively.

In connection with this complicated problem, the writer wishes to refer to the analytical data in the above tables, showing the relative numbers[3] of the total sugar molecules calculated.

	Morning	Afternoon	Fluctuation %		Morning	Afternoon	Fluctuation %
July 9	4.2	5.0	29	August 19	5.0	6.1	22
July 10	4.3	4.0	-7	August 20	6.1	6.9	13
July 11	5.0	6.8	36	August 23	6.7	6.8	1
July 12	6.6	6.1	-7	August 24	6.0	7.8	30

As will be clear from this table, the total sugar molecules generally increase (up to more than 30%) in the afternoon like the osmotic value, although the rate of their increase does not agree completely with that of the osmotic value. Furthermore it is to be noted that the relative number of the total sugar molecules is larger in August than in July in accordance with the higher osmotic value. Although these results are not sufficient to clear up the relations between the sugar content and the osmotic value, we can recognize à great significance of sugars as osmotically effective substances at any rate.

SUMMARY

1. In the leaves of *Polygonum sachalinense*, the osmotic value at incipient plasmolysis, the water content, sugars and starch were determined before

[1] Cited by WALTER (1931, p. 49 ff.).

[2] Cited by WALTER (1931, p. 32).

[3] Since the molecular weight of sucrose is 1.9 times greater than that of glucose, the relative number of the total sugar molecules is obtained by the sum of the percentage of simple sugars multiplied by 1.9 and that of sucrose.

sunrise and in the afternoon on successive days under rather changeable climatic conditions on Mt. Hakkôda.

2. The osmotic value at incipient plasmolysis was higher (up to nearly 30%) and the water content smaller in the afternoon than in early morning. Moreover, a parallelism in the rate of increase of the osmotic value and that of decrease of the water content was clearly recognized.

3. This fact shows that the water deficit induces a new formation of osmotically effective substances. Although the cause of the diurnal increase of the osmotic value in the normal state of the cell is attributed in the main to the water deficit, there is no doubt that the new formation of osmotically effective substances plays an important rôle in it.

4. The osmotic value at incipient plasmolysis, accordingly the amount of osmotically effective substances, and the water deficit recovered to an almost definite status every morning.

5. It was noticed that the rise of the osmotic value increased generally in accordance with the relative number of the total sugar molecules. There is no doubt about the great rôle of sugars played in the osmotic value of cell sap.

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LIMNODRILUS GRANDISETOSUS, NOV. SP., A FRESHWATER OLIGOCHAETE.

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(With 5 text-figures and Pls. XIII–XVII).

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Many of the specimens of the species under discussion were collected in the summer of 1929 from the firm sandy mud of streamlets and pools adjacent to the river Arakawa, which is the upper portion of the river Sumida, Tôkyo. They live usually at a depth of more than 20 cm. When collecting them I could not determine whether or not they congregate and swing their posterior parts, owing to the muddy water in the locality, but actually they were found apart from each other as slender reddish threads apparently clinging tenaciously to the solid mud when broken. After the mud was removed, they coiled up at first and then began to congregate together as usual in culture dish. The species here dealt with is noticeably different, however, from other Japanese species of *Limnodrilus* found hitherto in the shallow water of gutters and ditches, — namely *L. gotoi*, *L. willeyi*, and *L. motomurai* — in having no envelope; this is composed of a slimy secretion from the body and of fine mud particles, and covers the anterior half of the body. These worms creep into sandy mud as quickly as other species do into the mud of ditches.

EXTERNAL FEATURES. The worms are filiform, very long in proportion to their thickness, the hinder portion in particular being extremely thin and thread-like. The length of a well-narcotized, sexually-mature specimen is over 80 mm., the maximum thickness being less than 0.6 mm., even towards the anterior end, the main middle portion being less than 0.45 mm. The prostomium is roughly triangular, with a bluntly rounded anterior end. Segments I–V consist of shorter anterior and a longer posterior annuli (Pl. XIII, 1). The clitellar portion is somewhat swollen giving a slender fusiform appearance to the anterior end of the body. The segments, especially the post-clitellar, are notably long in proportion to their thickness, the length generally measuring more than twice the thickness. Therefore, the number of segments is comparatively small, 85–95 having been counted

in most cases.

SETA-BUNDLES (Pl. XIII, 3 and 4, and Pl. XIV, 7). In each segment, four seta-bundles are arranged in a setal zone at about two-sixths or three-sevenths of the respective segment length from its posterior termination. The position of the seta-bundles, especially the ventral ones, is nearer the median plane of the body in comparison with those in other Japanese species of *Limnodrilus*.

While the worms are immature, generally in the pre-clitellar segments two setae form a bundle, but when they are full-grown one or two incomplete ones are frequently added, and sometimes even four complete setae may be counted. In the post-clitellar segments, generally one, rarely two complete setae are found in a bundle.

SETAE (Fig. 1). All the setae are of the two-pronged sigmoid type, the nodule being situated at a distance less than one-third of the setal length from the distal end. The prongs are generally more or less worn out, sometimes being sharply pointed. In this species, however, it is apparent that the upper prong is naturally longer and stouter than the lower.

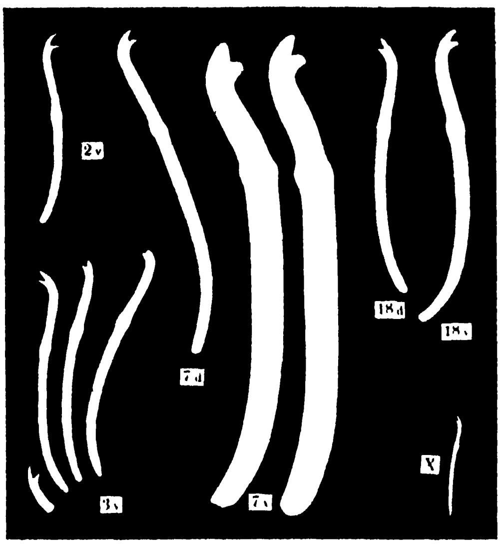

Fig. 1. Setae. ×350. 2, 3, 7, 18 and X--seta or seta-bundle in Segments II, III, VII, XVIII, and the last, respectively; d--dorsal setae, v-- ventral setae, 2--a young seta.

The ventral setae in Segments IV–X are characterized, in particular, by their discordant length and stoutness in proportion to those of the

corresponding dorsal setae in a segment, though usually the ventral setae are larger than the corresponding dorsal ones, even in other species of Tubificidae. This condition of the setae is one of the most remarkable characteristics of the present species, and this is the reason why I prefer *grandisetosus* as the specific name.

In Segments II and III, the dorsal and ventral setae are of the same type and are small, measuring only about from 75 μ to 87 μ long. The dorsal setae becoming larger gradually with the increase in the number in order of the segment, measure about 125 μ or more in Segment VII, and then begin to decrease towards the last segment in which they measure only 40 μ or less. The stout ventral setae beginning in Segment IV, increase in length towards Segment VII from 115 μ to 180 μ, and then decrease posteriorly, a transitional size and feature between normal and stout being found in Segment X. The ventral setae are absent in Segment XI. In the post-clitellar segments the dorsal and ventral setae are again of the same type.

Body Wall. In sexually-mature specimens, the clitellum extends from the beginning of Segment XI to the posterior end of Segment XII.

In the pre-clitellar portion, the body wall is generally thin, measuring from 15 μ to 30 μ in thickness when the body extended, but when fully contracted it measures 40 μ or more. In the post-clitellar portion, especially in the middle, the body wall is comparatively thick, measuring from 25 μ to 60 μ, according to the difference in thickness of the peritoneal and longitudinal muscle layers. No gland cells are found in the extra-clitellar hypodermis, this characteristic being probably correlated with the absence of a muddy envelope around the body.

Septa (Pl. XIII, 1, and Pl. XV, 12). The septa begin with the inter-segment III/IV. Those which are found at the inter-segments III/IV–IX/X are noticeably thick, and only the foremost three septa and Septum VIII/IX show a funnel-shape, the remainder being exposed nearly vertically. The septa at the inter-segments X/XI and XI/XII are very thin, and when the genital organs are fully developed they show also a funnel-shape.

Septal Sacs (Pl. XIV, 6). These are small and are attached to the posterior faces of Septa VI/VII and VII/VIII on both sides of the ventral vessel just ventral to the intestine. It is worthy of note that in an immature specimen I was able to find other three pairs of well-developed septal sacs attached to Septa III/IV–V/VI in the same position as those mentioned above. Therefore, it may be considered that the septal sacs of the present species develop in relation to Septa III/IV–VII/VIII, but when the worms

are young the anterior ones are formed before the posterior ones begin to develop, and on attaining sexual maturity the anterior ones disappear, the posterior ones remaining, but their size probably diminishes.

Buccal Cavity and Pharynx (Pl. XIII, 1 and 2). The dorso-ventrally flattened, thin-walled buccal cavity begins to be spacious towards Segment II and is transferred to the pharynx where it is clearly distinguished from the former by the presence of a ciliated, thick, ventral wall. The outline of the pharynx is fusiform as a whole having its maximum width at the middle of Segment III. This most widened portion is semi-circular in cross section, with a ∿-shaped lumen, the walls, thin lateral, thick dorsal and ventral being ciliated, and measures dorso-ventrally about one-fourth and laterally one-third of the diameter of the segment.

Near the posterior end of the pharynx a thin-walled, median dorsal inlet is observable. This inlet spreads anteriorly for some distance along both lateral margins of the pharynx, having a horse-shoe shape as a whole. Pharyngeal gland cells are not very numerous, and are attached to the walls of the dorsal inlet separately or forming masses.

Oesophagus (Pl. XIII, 1 and 3). The narrowed posterior end of the pharynx continues to the oesophagus, after piercing Septum III/IV. The oesophagus is a slender tube, measuring about from one-sixth to one-eighth of the diameter of the segment, and terminating at the posterior end of Segment V. Chloragogue cells are usually found in Segment V attached to the dorsal wall of the oesophagus, but they are never so numerous as those seen in the structure of the intestine.

Intestine. It commences at the anterior end of Segment VI, together with a thickly developed layer of chloragogue cells surrounding all sides of the intestine. In Segment VI, the intestine proper, excluding the chlorago-gue layer, is still narrow and shows nearly the same structure (Pl. XIII, 4) as that of the oesophagus, but on entering Segment VII it begins to increase in diameter, and comes to measure about one-fourth of the diameter of the segment (Pl. XIII, 5, and Pls. XIV and XV). The intestine retains this state until the genital segments, including the ovisac, arep assed over. From that point the dimensions of the intestine increase more or less suddenly, measuring about from one-third to one-half of the diameter of the segment (Pl. XV, 16). If we add the chloragogue layer, the intestine reaches from two-thirds to three-fourths of the segment diameter, and when the intestinal canal is filled with food it may occupy nearly the whole space of the coelomic cavity.

Nephridia. I have not been able to find the anterior nephridia in

the present species: I mean the nephridia which are found in Segments VII and VIII in the other species of *Limnodrilus*.

The posterior nephridia commence in Segment XIII. They are developed only on the left side of the body. The convoluting tubule of each nephridium spreads nearly along the whole length of a segment, mainly on the left side of the ventral nerve cord, but sometimes traversing also to the right side. They open exteriorly just in front of the setal zone, inside the left ventral seta-bundle. The asymmetrical development of the nephridia may not be an unusual feature among Japanese species of Tubificidae, a left side occurrence in *Tubifex hattai* and a right side occurrence in *Limnodrilus motomurai* having been reported by the present writer.

SPERM-SACS. Both the anterior and posterior sperm-sacs are unpaired. The anterior one is confined to Segment IX, and the posterior one when fully developed reaches to the posterior end of Segment XIV.

SPERM-DUCTS (Fig. 2). The sperm-duct funnel is somewhat urceolate and opens widely into Segment X. The vas deferens turns in manifold windings in Segment XI, its greater part being pushed into the posterior sperm-sac by the developing ova. It is circular in cross section, and is nearly of uniform diameter all through, viz. 25–30 μ, with its lumen of about from one-half to five-sixths of the diameter. The lumen is wider in the anterior portion than in the portion near the atrium. Consequently, the wall of the vas deferens where exposed is thicker in the posterior portion than in the anterior.

The atrium as a whole is fusiform, receiving the vas deferens at the

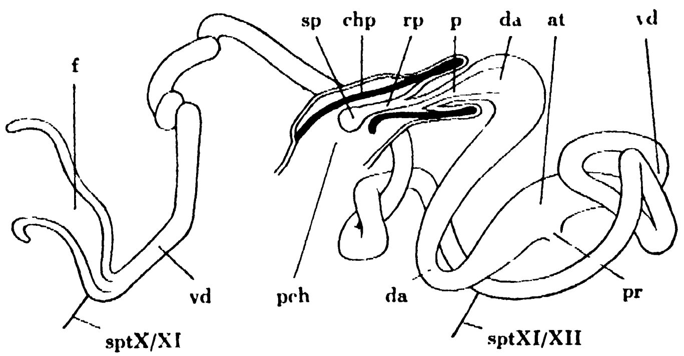

Fig. 2. Schematic representation of sperm-duct. ×100. Reconstructed. at — atrium, chp - chitinous penis sheath, da — duct portion of atrium, f — sperm-duct funnel, p — penis, pch — penial chamber, pr — opening of prostate gland to atrium, spt — septum, vd — vas deferens.

narrow end, and being continued to the atrial duct portion at the other end. The diameter at both ends is nearly of the same length as that of the vas deferens, the widest portion at the middle measuring 60–70 μ, as observed in specimens. The lumen is somewhat spacious, and shows a round, triangular, crescent, or irregular outline in cross sections.

The prostate gland is comparatively small, and irregularly shaped, being divided into several lobes, and is attached to one side of the widest portion of the atrium. The sphincter muscle surrounding the stalk of the gland is noticeably well developed.

Since the features of the atrial duct portion and of the penis are the most prominent criterion in arriving at a definition of the present species, it seems to me to be necessary to give somewhat detailed descriptions of them.

The atrial duct portion is nearly equal to, or more or less longer than, the atrium proper. It is also circular in cross-section all through, beginning with a narrow diameter nearly equal to that of the vas deferens. But this diameter then increases gradually towards the distal end and finally becomes wider than the widest portion of the atrium, measuring more than twice the diameter of the vas deferens. At the commencement of the duct portion, the non-ciliated, irregularly formed glandular epithelium of the atrium is transformed into a thin unicellular epithelium which encloses a circular lumen. The latter epithelium consists of cubical cells containing dense cytoplasm. This is then gradually transformed into a multicellular layer towards the distal end, and with the increase of the diameter of the duct portion, the lumen becomes wider and irregularly shaped again. The distal end of the duct portion is thickly invested with spirally arranged muscle fibres connecting with the musculature of the body wall.

The penis is short and is nearly conical, furnished with a protrusion which originates from its outer distal margin (Fig. 2, and Pls. XVI and XVII).

The penis proper is composed of two unicellular epithelia, outer and inner, enclosing an irregularly shaped penial lumen, and measures about 65 μ in length and about 74 μ in width at the proximal end. The multicellular epithelium of the atrial duct portion is again transformed, first at its distal end, into a unicellular epithelium, and continues to the inner penial epithelium, the diameter of the duct portion decreasing more or less suddenly. The inner epithelium consists of cubical, polygonal, and columner cells, while the outer epithelium consists of somewhat flatly formed cubical or polygonal cells. The space between the inner and outer epithelia is rather spacious at the proximal half, being completely filled with peri-

toneal cells, but it narrows gradually towards the distal end. The muscle layer investing the outer surface of the atrial duct portion intrudes also into the space between the inner and outer epithelia adhering to the inner, but this time it is a sphincter in character. At the distal end of the penis the margin of the opening of the penial lumen ought to be formed by a layer which is not differentiated into inner and outer. But, actually, at the level of about two-thirds of the penial length from the proximal end, the diameter of the penis becomes remarkably narrow, owing mainly to a sudden thinning of the outer epithelium. Thus, only the inner epithelium appears to form the marginal layer of the penial opening. Consequently, in serial cross sections, at that level the inside portion of the outer epithelium begins to disappear, only the inner epithelium being left. Then the inside portion of the inner epithelium begins to disappear, and finally only small outside portions of the inner and outer epithelia remain to form the base of the penial protrusion. The nuclei of the inner penial epithelium are generally smaller than those of the outer one and of the epithelium of the atrial duct portion.

The penial protrusion is a solid rod with a large swelling at its distal end. The whole protrusion is longer than the penis proper. The rod portion consists of elongated cells densely arranged, and the swollen portion of irregularly-shaped cells loosely arranged with several intercellular spaces.

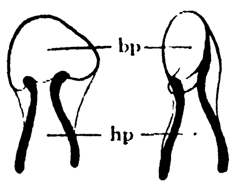

Fig. 3. Dorsal (left) and side (right) views of the chitinous penis sheath from fresh material compressed. ×100. bp blade portion, hp — handle portion.

The chitinous penis sheath (Figs. 2 and 3, and Pls. XVI and XVII) is shovel-shaped, consisting of a handle and a blade. Its thickness is almost uniform throughout the entire structure, measuring about 7 μ, even though the blade portion may appear to be thin when observed in a living specimen. The handle portion is circular in cross-section all through, narrowing towards its distal end, and is about twice as long as broad at its proximal end, where it measures about 85 μ. Nearly at the level of distal end of the rod portion of the penial protrusion, the blade portion begins to spread out laterally and anteriorly, originating from all the margins of the distal end of the handle portion, and showing a ventralwards concavity over the swollen portion of the penial protrusion. The blade portion is obtusely triangular, the narrow end being directed anteriorly and measuring about

95μ by 125μ. Its whole edge is terminated at the dorsal wall of penial chamber to which it is attached.

PENIAL CHAMBERS. Each organ is rather tubular and of nearly equal diameter, viz. $80-85 \mu$, with a spacious chamber only at its ental portion, where the maximum diameter measures about 135μ or more. The outer epithelium of the penis turns over distally at its proximal termination, and continues to the lining epithelium of the penial chamber, which is also uni-cellular, consisting of flatly-formed cubical or polygonal cells attached closely to the chitinous penis sheath. Nearly at the level of the base of the penial protrusion, the lining epithelium begins to separate from the chitinous sheath, and from that point becomes irregularly folded, but never comes again in contact with the sheath until the edge of the blade portion adheres to it in the dorsal region of the penial chamber. After proceeding antero-laterally, the chamber finally opens exteriorly on the longitudinal, ventral setal line at the middle of Segment XI. The outer surface of the penial chamber, especially its proximal portion, is thickly invested with spirally arranged muscle fibres.

SPERMATHECAE. Each spermatheca consists of two portions, saccular (Pl. XV, 12) and tubular (Pl. XIV, 10). Spermatophores are seen in the saccular portion. The tubular or duct portion is noticeably long, measuring more than twice the length of the saccular portion, and it opens exteriorly nearly at the middle portion of Segment X in front of, and a little inside, the ventral seta-bundle. In its natural position the wall of the saccular portion is fully distended, while that of the duct portion is very irregularly folded. The left and right spermathecae are usually arranged crosswise.

OVISAC. The posterior ovisac extends to the posterior end of Segment XIV.

In one specimen, an anteriorly-directed bulge from the anterior septum of Segment XI was observed, its anterior face having been entangled thickly with the commissural vessels of Segment X (Pl. XV, 13). In the present specimen, the coelomic space in Segment XI, as well as the posterior ovisac, was filled with ova in different developmental stages. The posterior sperm-sac, however, which ought to bulge out in a posterior direction also from the anterior septum of Segment XI, was not detectable, while the anterior sperm-sac was perfectly developed containing sperm morulae in several stages. Regarding this undeveloped posterior sperm-sac I cannot, of course, say decidedly whether it was beginning to form or had already degenerated. But from several other points, it appears to me that the present species may be protogynous, as an exceptional case

among many protandrous species of oligochaetes, and it also appears that the anteriorly directed bulge from Septum X/XI may be taken as an indication of the presence of an anterior ovisac, even though it may be only a temporary feature. In reality, I believe from examination of other specimens that the posterior sperm-sac develops directly from the probable anterior ovisac by its inversion into Segment XI, the commissural vessels being withdrawn into it.

Dorsal Vessel. The dorsal vessel divides at its anterior end just behind the prostomial ganglion, and runs posteriorly along the ventral surface of the brain. In Segment II, the vessel lies far apart from, but mid-dorsally to, the alimentary tract, and in Segment III it gradually approaches the median dorsal surface of the pharynx. In each segment posterior to III, the direction of the dorsal vessel when contracted varies in its course, but as this direction appears to change relatively to the position of each segment, it may be described in some detail. In Segment IV, the vessel runs posteriorly along the left dorsal side of the oesophagus, but later it turns to the right dorsal side. In Segment V, it runs at first along the median dorsal side of the oesophagus, then along the left dorsal, and finally turns suddenly to the right dorsal. In Segments VI and VII, it runs at first along the median or right dorsal side of the intestine, and then turns suddenly to the left dorsal. In Segment VIII, at the level of origin of the intestinal hearts it runs along the left dorsal side of the intestine, but at the setal zone it begins to deviate strongly to the left. Then, turning suddenly to the right dorsal and entering the chloragogue layer, it comes very close to the endoderm. In fact, in the segments anterior to VIII, the dorsal vessel never reaches the endoderm, even if sometimes it enters the chloragogue layer. In Segment IX, the vessel turns gradually from the left dorsal side of the intestine to the right dorsal, or even to the right ventral side. In Segment X, it runs for the most part along the right or right ventral side of the intestine. In the segments anterior to X the dorsal vessel resumes, as a rule, the median dorsal position in relation to the alimentary canal when it pierces the septa, but in the case of Septum X/XI it maintains the position of the right ventral. In the segments posterior to X it continues to run along the right or right ventral side of the intestine, even when piercing the septa, sometimes approaching the right side of the ventral vessel or of the ventral nerve cord. It is worthy of note, in connection with most segments in the middle and posterior regions of the body, that the dorsal vessel is almost invested with a chloragogue layer, which is separately formed from that of the

intestine. This may probably suggest a peritoneal origin for the chlorago-gue layer.

SUPRA-INTESTINAL VESSEL. This vessel opens into the dorsal vessel at the anterior end of Segment VI. In Segments VI-VIII it lies usually on the median dorsal side of the intestine, being attached to the endoderm. After entering Segment IX, however, it begins to run along the right dorsal side of the intestine, though it may, but less frequently, turn to the left dorsal side. In the segments posterior to XI, the presence of the supra-intestinal vessel is very doubtful, being sometimes recognized only as swellings in the intestinal networks.

VENTRAL VESSEL. Each branch from the anterior end of the dorsal vessel runs anteriorly along either side of the prostomial ganglion, and after winding a few times it runs first ventro-posteriorly along the inside of the peripharyngeal nerve, then along the dorsal side of the ventral nerve cord, finally uniting with the opposite branch just in front of the setal zone of Segment VI and forming a single ventral vessel. The latter vessel, running posteriorly, becomes extraordinarily thin in Segment VIII, and at the middle of Segment IX it continues to the thickened portion of the ventral vessel which is made by the union of the intestinal hearts. The ventral vessel runs usually along the dorsal side of the ventral nerve cord, but in the segments posterior to X, when the dorsal vessel comes to the right side, it moves to the left.

COMMISSURAL VESSELS. The commissural vessels are almost coelomic in Segments II and III, being sometimes attached to the peritoneal layer of both septum and body wall. Each of them branches out from the dorsal vessel just at the posterior end of the segment and proceeds dorso-laterally and anteriorly, and after winding in a few convolutions it opens into the paired ventral vessel on the respective side also at the posterior end of the segment.

In Segments IV and V, each commissural vessel leaves the dorsal vessel just in front of the posterioly directed end of the septal funnel and proceeds dorso-laterally along the anterior face of the septum. After a short course, it turns ventrally and attains the ventral body wall on the respective side of the ventral nerve cord straight through the coelomic cavity. Then it makes several undulations, but is always attached to the peritoneum, and finally comes to the dorsal body wall at the setal zone just inside the dorsal seta-bundle, and again proceeds towards the ventral body wall straight through the coelomic cavity. After running parallel to the paired ventral vessels, the commissural vessels open to the latter on the

respective side just in front of the posterior septum. Therefore, in these segments, we find the dorso-ventral course twice running through the coelomic cavity, one at the setal zone and the other just in front of the posterior septum.

In Segments VI and VII (Fig. 4), each vessel branches out laterally from the dorsal vessel just in front of the posterior septum, and soon turning towards the dorsal body wall it reaches the junction between the septum and the body wall, inside the longitudinal, dorsal setal line. Then

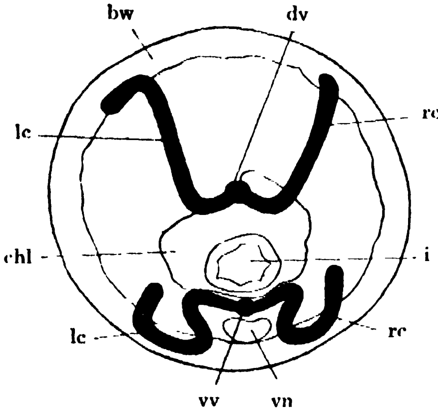

Fig. 4. Schematized cross section through the hinder region of Segment VII, to show the course of commissural vessels. × 100. Reconstructed. bw — body wall, chl — chloragogue layer, dv — dorsal vessel, i — intestine, lc — left commissural vessel, rc — right commissural vessel, vn — ventral nerve cord, vv — ventral vessel.

it makes several undulations being always attached to the peritoneum, and comes finally to the surface of the posterior septum near the ventral body wall. Then, running along the septal and ventral body walls for a short distance, it leaves the body wall and opens into the ventral vessel also just in front of the posterior septum.

In Segment VIII, the commissures are absent, as is usually the case.

In Segment IX, the commissural vessels branch out from the dorsal vessel which is right side to the intestine and are immediately attached to the outer surface of the anterior sperm-sac just in front of the posterior septum. After making antero-posterior undulations each leaves the sperm-sac for the dorsal body wall running along the surface of the posterior septum, but through the coelomic cavity. Then it again makes several undulations, becoming attached to the peritoneum of the body wall and coming finally to the respective lateral side of the entrance to the anterior

sperm-sac, from which point it runs directly towards the ventral vessel again through the coelomic cavity. The present commisures show neither the pulsation nor the pulsatory structure, which may be seen in those of *Limnodrilus gotoi* and *L. willeyi*.

In Segment X, the course of the commissural vessels is nearly the same as that in Segment IX, except that at the start from the dorsal vessel each enters the posterior sperm-sac. It passes the ventral side of the sperm-duct funnel before it opens into the ventral vessel.

In Segment XI, the course is also the same as that in Segment IX, but each vessel, at its start from the dorsal vessel supplies the posteriorly directed ovi-sac.

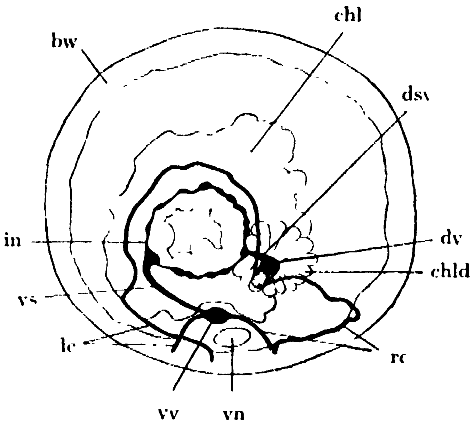

Fig. 5. Schematic representation of the posterior half of a segment at the middle region of the body, to show the course of the commissural vessels and the relation between the dorsal, ventral, dorso-supra-intestinal and the ventro-sub-intestinal vessels. × 100. Reconstructed. bw — body wall, chl — chloragogue layer investing the intestine, chld — chloragogue layer investing the dorsal vessel, dsv — dorso-supra-intestinal vessel, dv dorsal vessel, in — intestinal canal, lc — left commissural vessel, rc — right commissural vessel, vn — ventral nerve cord, vs ventro-sub-intestinal vessel, vv — ventral vessel.

In the segments posterior to XI, each commissure is remarkably fine, and is integumentary over most of its course. Each originates from the dorsal vessel just in front of the posterior septum, and enters the ventral body wall on the respective side of the ventral nerve cord at distance from the septum, the left one after proceeding around the intestine through the chloragogue layer, and the right one via the right ventral body wall (Fig. 5).

INTESTINAL VESSELS (Fig. 5). In a middle or posterior segment, usually only one dorso-supra-intestinal vessel is found at a level a little posterior to the setal zone, and only one ventro-sub-intestinal vessel a little anterior to it. They are apparently connected with the intestinal network, the presence of the supra-intestinal and sub-intestinal vessels being very doubtful.

REMARKS.

In the late autumn of 1910, I was able, on one occasion, to collect some specimens of a freshwater oligochaete from the sandy mud of a narrow canal, the water of which was supplied from the river Arakawa, and which ran through Ôji, then a northen suburb of Tôkyo. This canal was not found in 1929.

The worms were clearly characterized by their slenderness and depth of habitat in comparison with the other species of Tubificidae, which are commonly found in Japan. In an examination of fresh specimen under a microscope, the specially thickened anterior septa and disproportionately sized ventral setae attracted my attention. However, as the specimens were immature, at most only two setae were counted in each seta-bundle of pre-clitellar segments, and the chitinous penis sheath had not yet formed, so that time I believed rashly that the species did not belong to the genus *Limnodrilus*.

In June of 1929 I again made a collection searching principally for the species under discussion, and I was able to obtain a number of sexually-mature specimens from streamlets and pools in the catchment area of the Arakawa near the Akabane Bridge.

As already stated the present species is accurately distinguished from the other species of *Limnodrilus* by the smaller number of its setae, all of which are of the two-pronged sigmoid type; by the discordant difference in size between the dorsal and ventral setae in the pre-clitellar segments; by the absence of anterior nephridia; by the presence of posterior nephridia only on the left side of the body; by the special dilatation in the distal half of the atrial duct portion; by the presence of a penial protrusion attached to the apex of a short conical penis; and by the presence of a short shovel-shaped, chitinous, penis sheath.

A nearly similar shape of chitinous penis sheath was drawn and a small number of setae were described by STEPHENSON in 1929 in describing his *Limnodrilus sp.* from Upper Burma. Notwithstanding that his description is brief, it seems to be most probable that the Japanese and

Burmese species are closely allied, but as the former species differs from the latter, at any rate not only in the dimensions of the blade portion of chitinous penis sheath but especially in the proportions of the dorsal and ventral setae, which can never escape the eyes of any investigator, I wish to propose the new name, ***Limnodrilus grandisetosus***, for my present species, even though these characteristics may not be specific.

It may be noted here that in localities of the same depth ***L. grandisetosus*** may be found together with ***Branchiura sp.*** (it may be ***Br. sowerbyi*** BEDD., but I am quite uncertain about this at present), sometimes with ***L. gotoi*** HATAI, ***L. willeyi*** NOMURA, and even with ***Tubifex hattai*** NOMURA, especially after a flood, but ***L. grandisetosus*** has never been found in such shallow water as that of gutters or ditches, where several species of Tubificidae are abundantly found.

Finally, I will here give a summary of the distinguishing features of the present new species.

Limnodrilus grandisetosus, nov. sp.

Body filiform, remarkably slender; colour red or reddish brown; anterior septa comparatively thick; transverse striations due to septa are not seen in the posterior part; length 80–100 mm.; number of segments 85–95. Prostomium roughly triangular. Segments I–V biannulate, posterior annulus longer. Clitellum forming a complete ring and occupying Segments XI and XII, both entirely inclusive. Brain almost square, emarginate posteriorly. Setae sigmoid, bifurcate; most prongs worn out, but upper prong generally larger. Each seta-bundle consists of 2 or 3, sometimes of 4 setae in the anterior segments, of 2 or 1 in the middle segments, and of 1 in the posterior. The ventral setae in Segments IV–X disproportionately larger than the corresponding dorsal ones; for example, in Segment VII the largest ventral seta is about one and a half times as long and three times as wide as the largest dorsal. Two pairs of small septal sacs attached to the posterior faces of Septa VI/VII and VII/VIII on the ventral side of the intestine in sexually-mature specimens. Pharyngeal gland cells attached to the wall of the horse-shoe shaped dorsal inlet of the pharynx separately, or forming masses; oesophagus in Segment IV and V; intestine and chloragogues commence in Segment VI. Anterior nephridia absent; posterior nephridia only on the left side of the body. Prostate gland small, irregulary shaped. Atrial duct portion dilated distally, wider than the atrium or twice or more than twice as wide as the vas deferens. Penis

short, conical, with a penial protrusion. Chitinous penis sheath shovel-shaped, with a horizontally spread blade portion longer than the half of a tubular handle portion; the handle portion circular in cross-section all through, twice or less than twice as long as it is broad at the proximal end. Anterior sperm-sac unpaired. Both the posterior sperm-sac and ovisac reach the posterior septum of Segment XIV. Duct portion of the spermatheca twice or more than twice as long as the saccular portion.

Habitat — Catchment area of the river Arakawa, Tôkyo.

August 18, 1932.

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EXPLANATION OF PLATES.

ad ······ atrial duct portion
asp ····· anterior sperm-sac
br ······ brain
c ····· commissural vessel
cao ··· commissural vessel entangled in the outer surface of probable anterior ovisac
chl ····· chloragogue cell or layer
cs ··· chitinous penis sheath
d ······· dorsal vessel
db ······ dorsal seta-bundle
ds ······ distal swelling of penial protrusion
dsp ··· duct portion of spermatheca
easp ···· entrance to anterior sperm-sac
es ···· oesophagus
ff ······· follicular fundus of seta-bundle
fp ···· female pore
h ····· intestinal heart
i ····· intestine
if ······ interfollicular muscle
ipe ····· inner penial epithelium
li ···· connection between lateral line and interfollicular muscle
ll ······· lateral line
lm ······ longitudinal muscle fibres of body wall
lpc ····· lining epithelium of penial chamber
m ····· mouth
mp ····· male pore
mpp · middle portion of penial protrusion
n ······ ventral nerve cord
neph ·· nephridium
o ···· ovary
of ····· oviduct funnel
ope ···· outer penial epithelium
ov ··· ovum
p ···· penis
pch · penial chamber
ph ·· pharynx
phg · pharyngeal gland

ppp ··· proximal portion of penial protrusion
pr ···· prostomium
prg ····prostomial ganglion
psp ··· posterior sperm-sac
sc ····· commissural vessel in posterior sperm-sac
sf ···sperm-duct funnel
si ······supra-intestinal vessel
sm ···spiral muscle fibre
sml··· sperm morula
spp···· spermathecal pore
sps ··· saccular portion of spermatheca
spt ···· septum
spth ···spermatheca
ss ······septal sac
st ···· seta
sv ··· sub-intestinal vessel
t ··· ···testis
tm ····transverse parieto-vaginal muscle
v ····ventral vessel
vb ····· ventral seta-bundle
vd ·· vas deferens
vs ····ventro-sub-intestinal vessel
I, II, III, IV, IX, X, XI, XII ·· number of segment
III/IV, IV/V, V/VI, VI/VII, VIII/IX, IX/X ··number of septum

Plates XIII–XV. Sections of body. × 100. Microphotos.: —

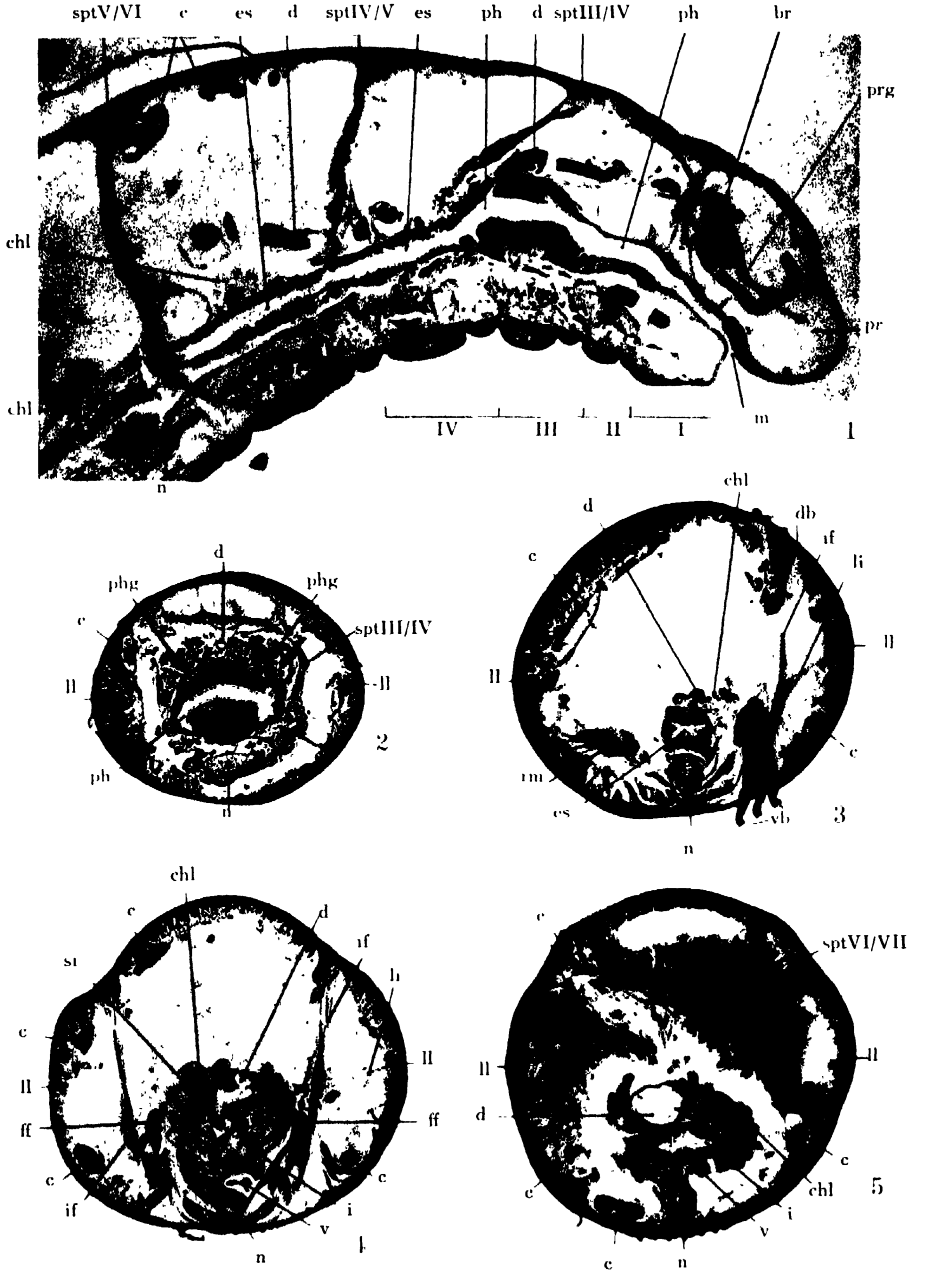
1. Median longitudinal section through Segments I–V.
2. Cross section of Segment III through widest portion of pharynx.
3. Cross section through setal zone of Segment V.
4. Cross section through setal zone of Segment VI.
5. Cross section through Septum VI/VII. On left side, a fibrous tissue, which may be nervous, is seen connecting lateral line with septum.
6. Cross section through posterior face of Septum VI/VII.
7. Cross section through setal zone of Segment VII.
8. Cross section through anterior portion of septal funnel VIII/IX.
9. Cross section through posterior portion of septal funnel VIII/IX.
10. Cross section through middle portion of Segment X.
11. Cross section through posterior face of Septum X/XI.
12. Longitudinal section of Segments X and XI through genital pores, nearly along longitudinal, ventral setal line.
13. Cross section through anterior face of Septum X/XI.
14. Cross section through middle portion of Segment XI.
15. Cross section through posterior portion of Segment XI.
16. Cross section nearly through setal zone of a segment in middle region of body.

Plates XVI and XVII. Sections through the atrial duct portion, penis, and penial chamber. Microphotos.: —

A. Horizontal section of penis, from a longitudinal section of body. ×230.

1-26. From 10 μ serial cross sections of body. The numerical order of Figures directly denotes order of serial sections from posterior to anterior. ×300.—

1. Longitudinal section of atrial duct portion. The left end continues to the penis.
3. Cross section nearly through distal end of atrial duct portion.
7. Cross section through proximal portion of penis.
9. Cross section through distal portion of penis. Outer penial epithelium is still present.
10. The same as 9. Outer penial epithelium is beginning to disappear at its median side.



E. Nomura: *Limnodrilus grandisetosus*, nov. sp.

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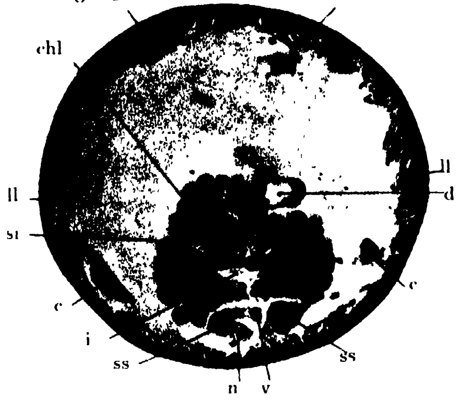

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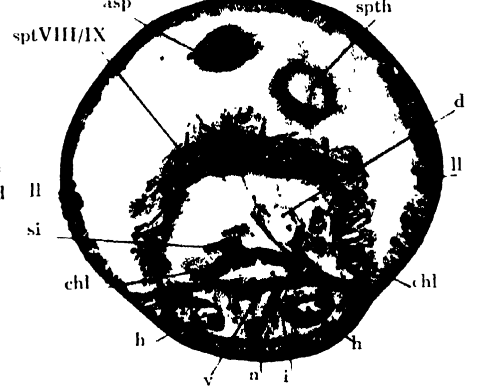

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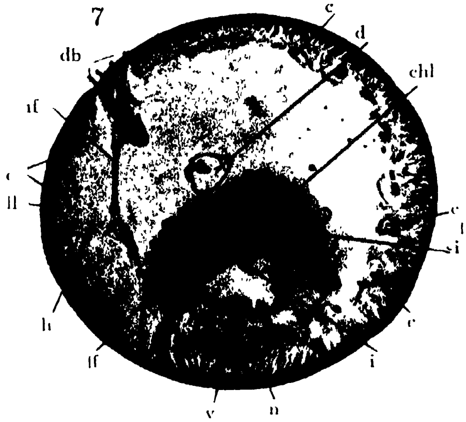

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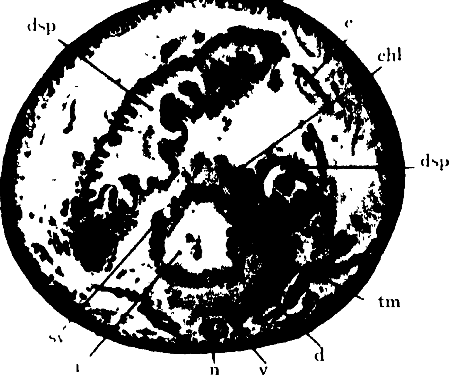

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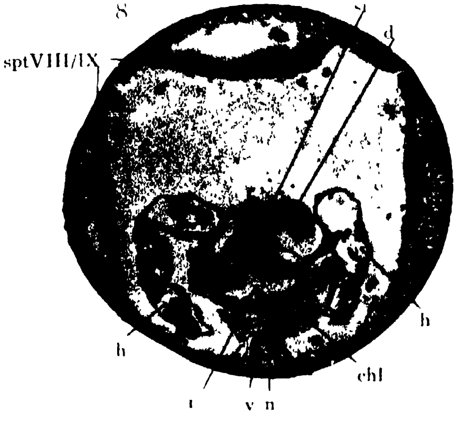

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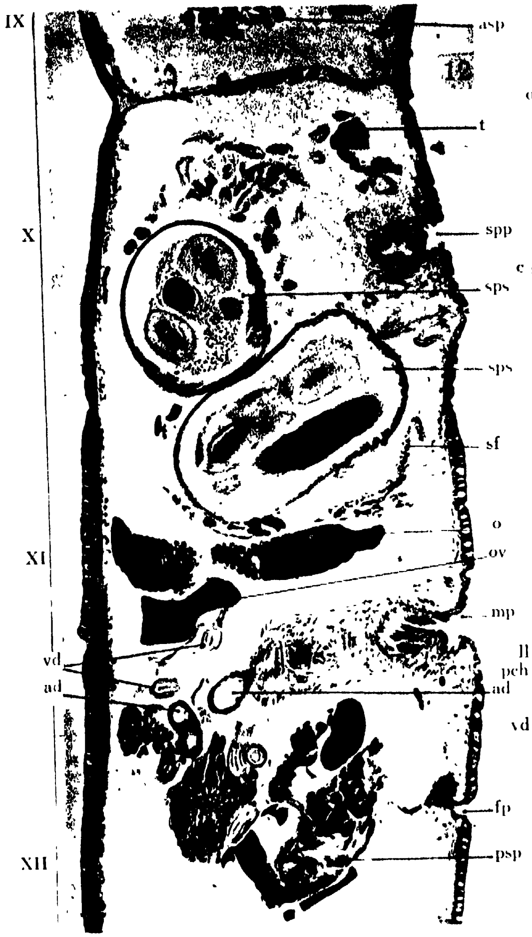
E. Nomura: *Limnodrilus grandisetosus*, nov. sp.



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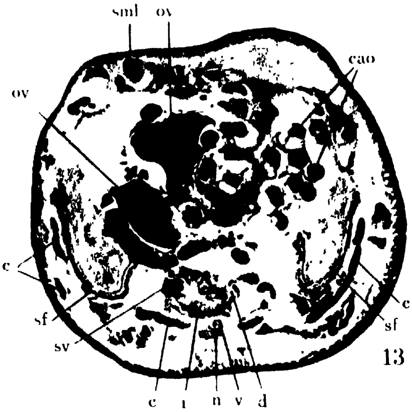

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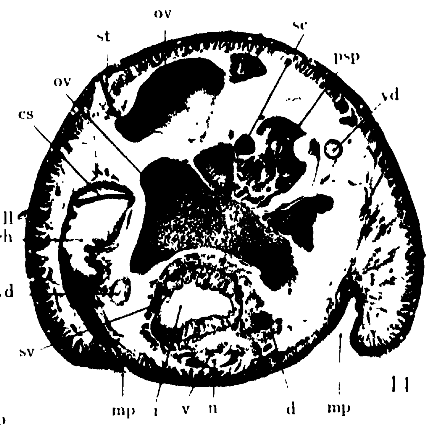

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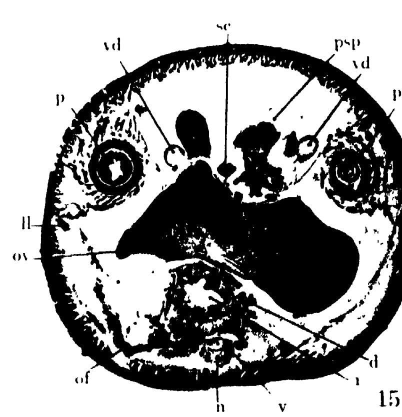

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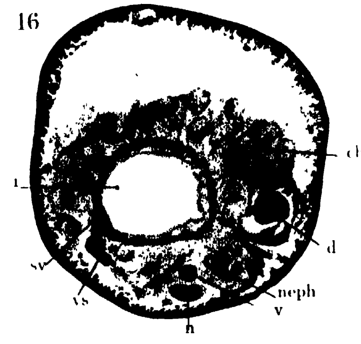

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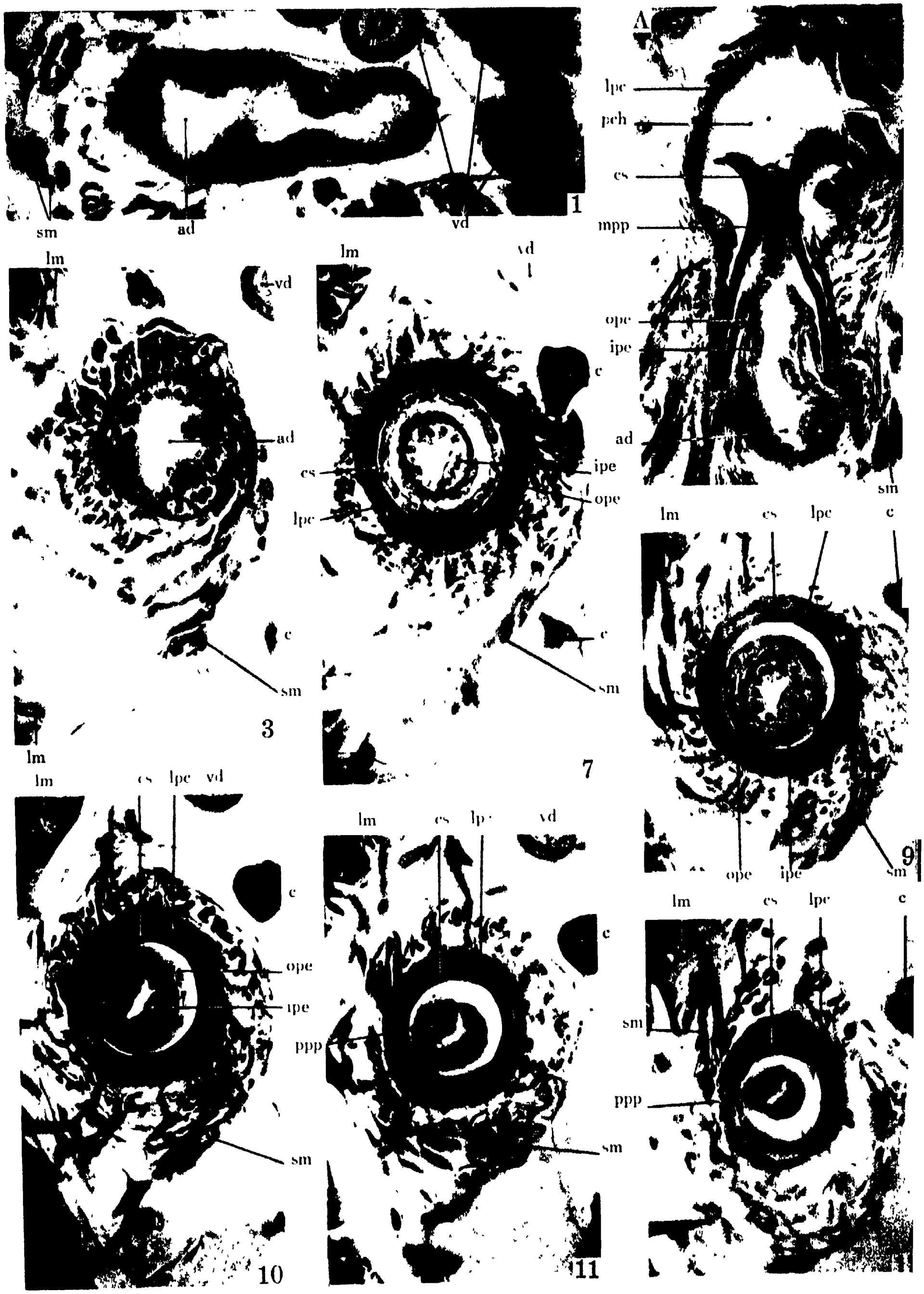
E. NOMURA: Limnodrilus grandisetosus, nov. sp.



E. NOMURA: Limnodrilus grandisetosus, nov. sp.

E. NOMURA: *Limnodrilus grandisetosus*, nov. sp.

11. Cross section nearly through distal end of penis. Outer penial epithelium has disappeard from nearly whole circumference, portion at base of penial protrusion only remaining.
12. The same as 11. Outer penial epithelium remains only, covering basal portion of penial protrusion. The diminution of diameter of chitinous penis sheath becomes somewhat acute.
15. Cross section through middle of rod portion of penial protrusion. The more the chitinous sheath decreases in diameter, the more spacious the penial chamber.
17. Cross section through distal end of rod portion of penial protrusion. The chitinous sheath becomes narrower and the blade portion begins to spread out from its ventral side.
19. Cross section through the swollen portion of the penial protrusion. The blade portion spreading laterally and dorsally forming a roof over distal end of penial protrusion.
21. The same as 19. The blade portion spreading nearly horizontally, showing a ventralwards concavity.
23. Penial protrusion has disappeared. In 19, 21, and 23, the lateral edges of blade portion are attached to lining epithelium of penial chamber.
25. Anterior edge of blade portion terminating at dorsal wall of penial chamber, which is proceeding antero-ventrally towards ventral body wall.
26. Chitinous penis sheath has disappeared and ventral cavity of penial chamber is approching ventral body wall.

ON THE CURVATURE OF THE CULM OF *SASA KURILENSIS*.[1]

By

KATSUTARÔ MIURA.

Biological Institute, Tohoku Imperial University, Sendai, Japan.

(With 11 Text-figures)

This brief note gives the results of studies of the curvature of *Sasa kurilensis* MAKINO et SHIBATA (syn. *Pseudosasa kurilensis* MAKINO) undertaken during my stay at the Mt. Hakkôda Botanical Laboratory during the summers of 1930 and 1931.

As is well known, this plant is characterized by the curving at the base of the culm, and from this peculiar property the Japanese name "Nemagari-dake"[2] is derived.

In spite of the rather wide distribution of this plant in northern Japan the reports at our disposal are very incomplete concerning its morphological and also its ecological properties, although Prof. Y. OKADA[3] of this labora-

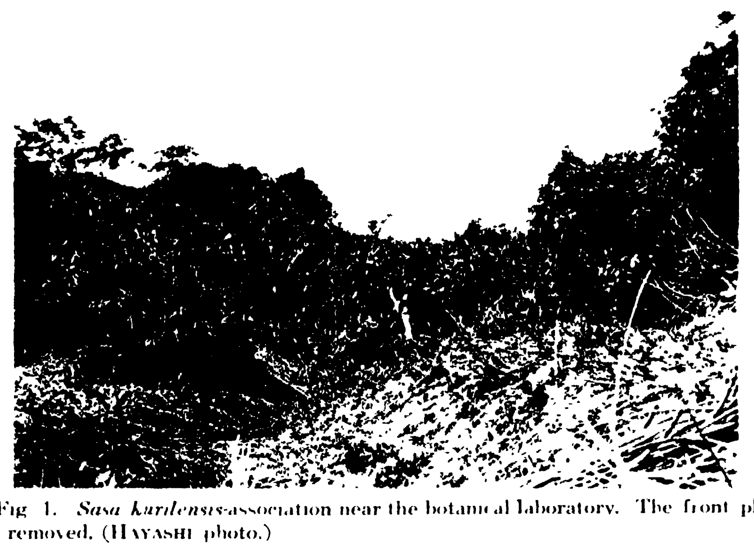


Fig. 1. *Sasa kurilensis*-association near the botanical laboratory. The front plants were removed. (HAYASHI photo.)

[1] Contributions from the Mt. Hakkôda Botanical Laboratory, No. 17.

[2] It means after all a sasa plant which has a curved culm.

[3] OKADA, Y. Contribution to the Knowledge on the Soil Microflora of *Pseudosasa*-association. I. Sci. Rep., Tohoku Imp. Univ., Ser. IV, Vol. 6, p. 149. 1931.

tory has recently published an interesting note on the microflora in soils on which the sasa-shrub grows. In studying these, the writer's aim was first of all to investigate the development of the rhizome and to examine the curvature of the culm, from the point of view of the relation between this curvature and the inclination of the ground.

Predominating over all others, this sasa-community is widely distributed round the botanical laboratory on Mt. Hakkôda, which stands about 900 metres above sea-level (Fig. 1). Among the numerous shrubs which are found in the vicinity, we may mention *Sorbus Aucuparia*, *Ilex Sugeroki* subsp. *brevipedunculata*, *Viburnum furcatum*, *Daphniphyllum humile*, and, furthermore, we find scattered here and there such species as *Trientalis europaea*, *Maianthemum bifolium*, etc.

The vertical distribution of the sasa-community on Mt. Hakkôda reaches generally a height of about 1300 metres above sea-level. Above this height, the plant becomes gradually rarer. Our botanical laboratory, therefore, is surrounded by a luxuriant growth of the plant, thus furnishing an abundance of materials for its study.

I) The Development of the Rhizome.

The rhizome of the plant usually grows parallel to the soil surface, whether the ground is inclined or not. On attaining a certain length the growth of a rhizome ceases, a new rhizome coming from the last node, or sprouting from a node of the culm under ground. The branching of the rhizome is, therefore, of three different kinds as shown in figures 2, 3 and 4.

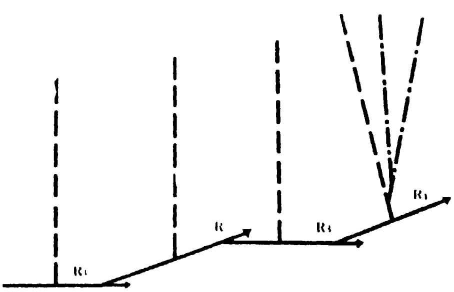


Fig. 2. Growth and branching of rhizomes and culms of type I (schematic). ---- Rhizome (R), — Dead culm, —·— Culm with leaves.

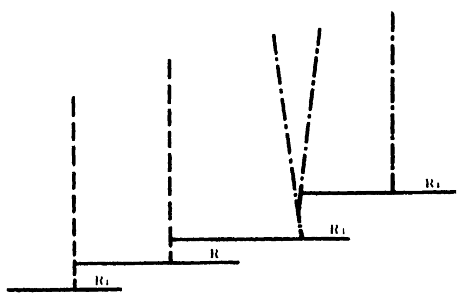


Fig. 3. Growth and branching of rhizomes and culms of type II (schematic).

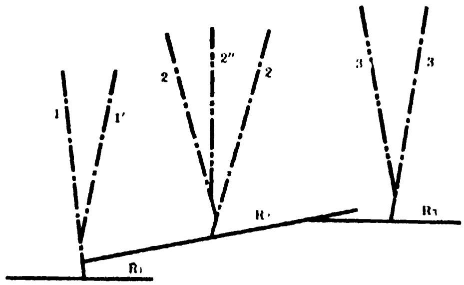


Fig 4. Growth and branching of rhizomes and culms of type III (schematic)
—— Rhizome (R), - - - — Old culm, - - Young culm with leaves.
See Fig. 5, a and b

1. The first type. The mother rhizome R_1 sprouts first, followed by rhizome R_2, which later produces another rhizome R_3 and so on, constituting a long series of these (Fig. 2).

2. The second type. The mother rhizome R_1 first sprouts a culm, from which rhizome R_2 is derived, and in this way the propagation of the plant is carried on (Fig. 3).

3. The third type. In this case, the propagation of the plant is effected by the combination of the first and the second methods, as is clearly shown in figure 4 and photographs (Fig. 5, a and b).

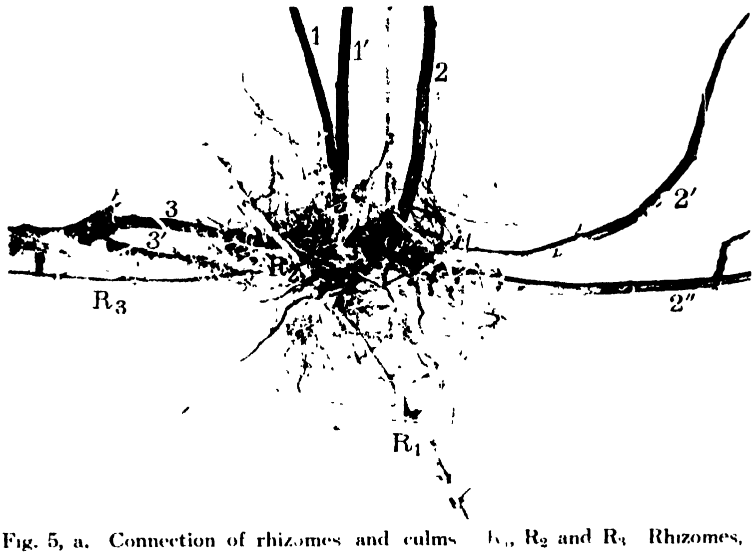


Fig. 5, a. Connection of rhizomes and culms R_1, R_2 and R_3 Rhizomes, 1, 1', 2, 2', 2'', 3 and 3' Culms. (HAYASHI photo.)

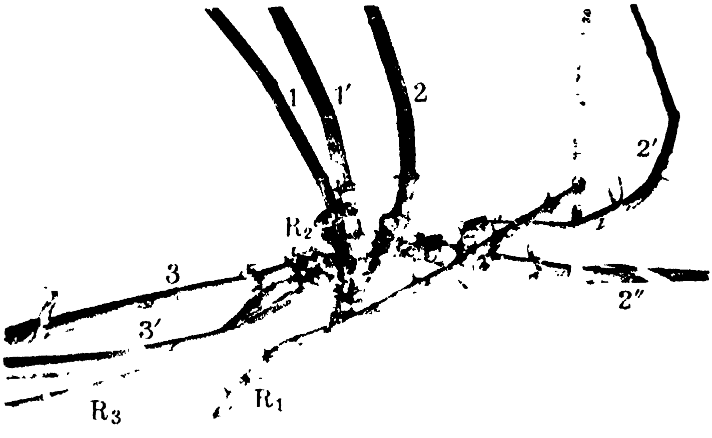


Fig. 5, b. Connection of rhizomes and culms. Same as Fig. 5, a, roots removed. R_1, R_2 and R_3 Rhizomes, 1, 1′, 2, 2′, 2″, 3 and 3′ Culms. (HAYASHI photo.)

Fig. 6 The curvature of the culm of a young shoot (HAYASHI photo.)

II) The Curvature of the Culm.

The curvature of this plant which always takes place at the base of the culm (Fig. 6), can be divided into two types, namely; that which curves concavely only once, and that which curves twice, first concavely and then convexly.

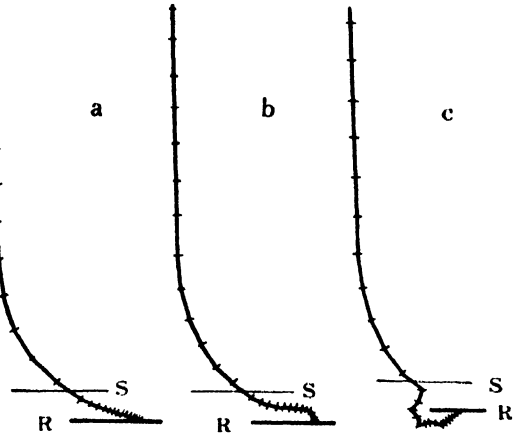


Fig. 7. The 1st type of curvature of culms. S—Surface of soil, R- -Rhizome.

(I) The first type (Fig. 7).

In this type, the curvature takes place at the base of the culm, presenting as a whole a smooth concave curvature (Fig. 7, a). The measurement of each curvature between two adjoining nodes shows that the curvature of the lower nodes, with the exception of some initial ones, is remarkably large, but becomes smaller at the upper nodes. This relation is seen distinctly in figure 8, a. In this graphical figure, the degree of curvature is taken as the ordinate — denoting the supplementary angle of the curvature of each node — and the length of the culm from the first node as the abscissa. Sometimes, the basal part of the culm, which consists of the diminished nodes, grows first vertically and then runs horizontally (Fig. 7, b and Fig. 8, b). As an exceptional case, the base of the culm has an irregular growth under ground, avoiding stones or other obstacles in the soil (Fig. 7, c).

(II) The second type (Fig. 9).

In this type, several initial nodes curve together concavely under ground, while only one node (Fig. 9, c, d, e) or two upper adjoining ones curve together convexly near the surface of the soil. In the latter case, either

one node curves at the surface of the soil, and the other under ground (Fig. 9, a), or the surface of the soil lies between the two curved nodes (Fig. 9, b).

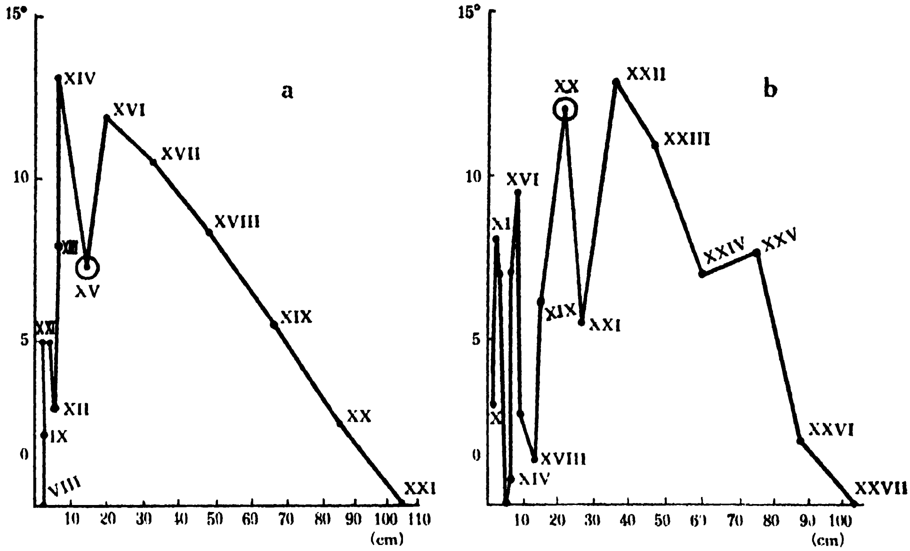


Fig. 8. Graphical figures of the curvature of the culm of Fig. 7, a and b. Roman numerals denote the number of the nodes and o shows the node which appeared first on the ground. In b the first figure X denotes the first node where the normal curvature begins.

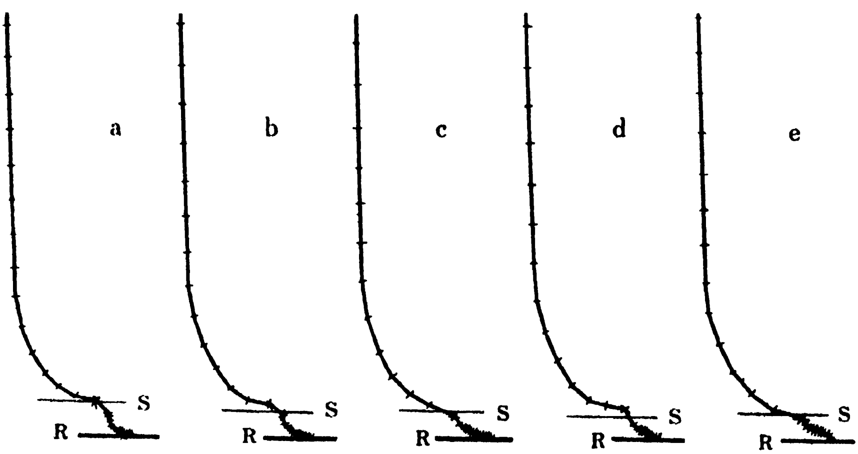


Fig. 9. The 2nd type of curvature of culms.
× denotes the node which curved convexly. S—Surface of soil, R—Rhizome.

This is more clearly shown in the graphical figures (Fig. 10, a, b, c, d). In these figures not only is the degree of curvature of each node given but also the distance of each node from the first node, as explained above.

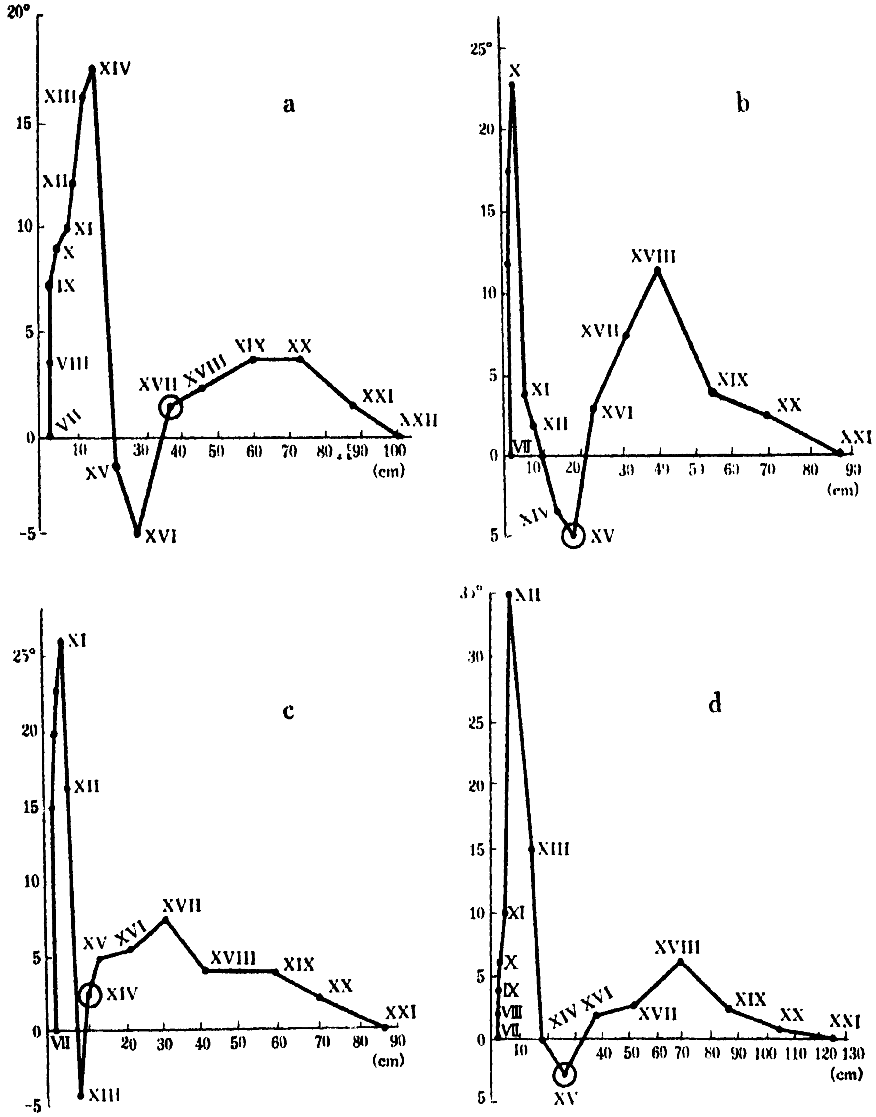


Fig. 10. Graphical figures of the curvature of the culms which are shown in Fig. 9, representing the angle of curvature of each node on the ordinate. For explanation of the figures, see Fig. 8. The node marked under the abscissa denotes the convex one.

The type which curves twice may be seen as a special case of the first type in which the curvature takes place only once under ground. This assumption is based on the following facts. Of a total of 25 separate specimens of the young shoots which were examined in the early summer of 1931, 22 belonged to type I and only 3 to type II. The same measurement was made of 67 adult plants. In this case, 30 belonged to type I, and the remaining 37 to type II. This increase in type II, which has the second curvature near the ground, confirmed the writer's conclusion that the curvature of the plant has arisen secondarily from external causes, being possibly caused by the deep snow of the first winter of its growth. In connection with this suggestion, it may be noted that the degree of the second curvature on the ground is always greater than that of the first curvature under the ground.

Tables 1 and 2 show the results of the curvature measurements of

TABLE 1.

The degree of curvature and the length of the curved part of an adult culm of type II.

No. of individuals	No. of node, at which the curvature begins	Length of a culm to the first curvature (cm)	No. of node, at which the curvature ends	Length of a curved part (cm)	Average degree of each curvature	Inclination of the ground (degree)	Note
1	10	1.6	25	79.5	172	46.5	The basal part of the culm grows vertically.
2	8	1.9	20	82.6	173	,,	
3	9	1.7	22	51.4	171	,,	
4	6	2.1	22	114.7	172	,,	
5	9	2.1	26	86.1	173	,,	The basal part of the culm grows vertically.
6	9	2.4	27	111.6	174	,,	
7	5	1.1	24	103.4	174	,,	
8	10	1.8	23	61.1	173	,,	The basal part of the culm grows vertically.
9	9	1.3	26	102.9	173	,,	
10	8	1.7	24	89.8	174	,,	
11	3	0.4	18	74.0	173	,,	

TABLE 2.

The degree of curvature and the length of curved part of an adult culm of the type I.

No. of individuals	No. of node, at which the curvature begins	Length of the culm to the first curvature (cm)	No. of node, at which the curvature ends	Length of a curved part (cm)	Average degree of each curvature	Inclination of the ground (degree)	Note
12	9	1.9	21	60.1	176 -182 169	46.5	
13	9	2.0	23	66.4	171 -185 171	,,	
14	9	2.3	25	100.6	174 -186 171	,,	
15	5	1.0	19	65.1	173 -187 170	,,	
16	15	4.2	28	115.8		,,	The basal part of the culm grows vertically.
17	8	1.1	28	103.5		,,	
18	7	1.6	24	102.8	177 -187 172	,,	
19	5	0.9	20	91.8	177 -185 170	,,	
20	5	0.7	18	53.1	170 -182 172	,,	
21	7	1.0	18	71.5	175 -182 177	2.5	
22	7	1.2	21	80.8	154 -184 174	,,	
23	8	1.3	18	50.3	163 -182 175	,,	
24	7	1.6	19	77.9	166 -183 176	,,	The basal part of the culm grows vertically.
25	8	1.0	20	69.0	176 -183 173	,,	,,
26	7	2.5	21	86.5	169 -183 177	,,	
27	7	0.9	20	69.1	160 -181 175	,,	

No. of individuals	No. of node, at which the curvature begins	Length of the culm to the first curvature (cm)	No. of node, at which the curvature ends	Length of a curved part (cm)	Average degree of each curvature	Inclination of the ground (degree)	Note
28	11	3.1	26	81.3		2.5	The basal part of the culm grows vertically.
29	6	0.8	18	69.2	170 -195 175	,,	,,
30	7	1.0	21	81.5	166 -184 177	,,	
31	8	1.0	20	60.5	170 -183 175	,,	The basal part of the culm grows vertically.
32	9	1.3	25	65.9	175 -182 174	,,	
33	7	1.6	20	102.9	168 -182 177	,,	
34	8	1.1	25	70.4		,,	The basal part of the culm grows vertically.
35	9	1.8	21	54.0	160 -189 175	,,	,,
36	7	0.8	21	69.2	167 -184 175	,,	
37	7	1.8	19	64.7	163 -183 175	,,	The basal part of the culm grows vertically.
38	9	2.2	21	67.1	178 -181 176	,,	,,
39	7	1.9	16	66.1	162 -193 174	,,	
40	7	1.3	20	69.9	167 -188 175	,,	

culms which belong to types I and II. There is practically no difference between these two types, except in the number of the curvatures, as stated above. In general, the curvature begins at the 7-th or 8-th node 1 to 2 cm in length, and the culms become straight again at about the 20-th node, so that the culm curves on about 13 nodes. The degree of curvature between two adjoining nodes is 172° in type I on the average. This measurement in type II is 169° at the first curvature, and 172° at the second, while the convex curvature between them showed 2° on the average.

The difference in curvature between the culm of an adult plant and

that of a young shoot was then examined. According to UCHIDA[1], the inherent curvature has already taken place at the time of the young shoot, having already curved convexly towards the running direction of the rhizome, afterwards, curving more and more convexly owing to mechanical or other external actions. The fact that the curvature of the culm almost always takes place in the growing direction of the rhizome has also been confirmed by the present writer.

To account for the difference in culm curvature between the adult plant and the young shoot, 29 specimens of the plant and 15 of the shoot were collected in one place, and the degree of curvature of the culm in all these plants was measured with a graduator. They all belonged to type I, and therefore curved convexly near the surface of the soil. Each culm was measured by the following method, as will be seen in figure 11.

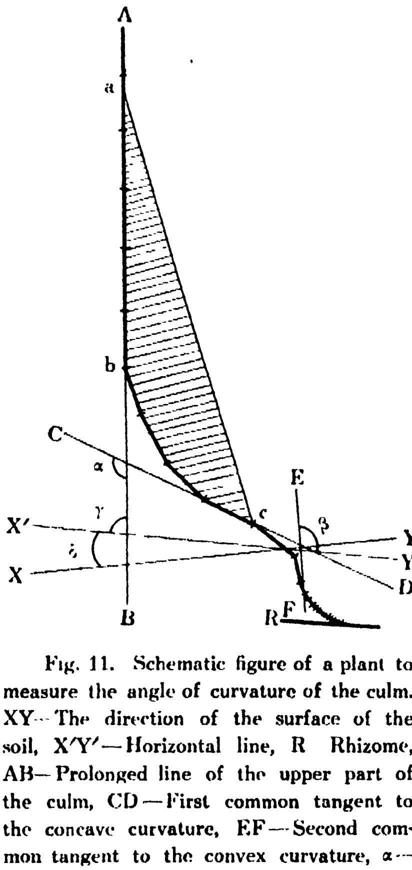


Fig. 11. Schematic figure of a plant to measure the angle of curvature of the culm. XY—The direction of the surface of the soil, X′Y′—Horizontal line, R Rhizome, AB—Prolonged line of the upper part of the culm, CD—First common tangent to the concave curvature, EF—Second common tangent to the convex curvature, α—Angle of intersection of AB and CD, β—Angle of intersection of CD and EF, γ—Angle of intersection of AB and X′Y′, δ—Inclination of ground, abc—A culm.

From the results of these measurements we obtained, on the average, the following values. α of the plant is about 140° and β about 159°, while α and β of the shoot are 138° and 167°, respectively; therefore, α of the plant is nearly equal to α of the shoot, but β is 8° greater in the shoot than in the plant. From these results of the measurements of α and β, it is clear that the adult plant curves more distinctly on the surface of the ground than does the young shoot.

To ascertain the difference in bending between the young and the

[1] UCHIDA, S. Nemagari-dake no Kenkyû. (Studies on *Sasa kurilensis*), in Japanese. Scientific Researches of the Alumni Association of the Morioka Agr. College. Vol. I, pp. 33–45, 1923.

adult plants the writer made other measurements.

If γ denotes the angle of intersection AB and X'Y' (the horizontal line), then it shows the angle of inclination of the culm to the horizontal surface. The γ-value of a young shoot is nearly equal to 90°, while according to the average of 47 individuals the value of an adult plant is 73°. For measurement, the writer used only those culms of adult plants, which sprouted last year, since they had undergone only one winter. We are justified in being convinced that the γ-value will decrease with succeeding years. From the results of these measurements, it seems to me that the culm of the plant will bend to the ground more and more with age. This may with certainty be attributed to the mechanical action of snow, which here lies on the mountain till the late spring of each year.

We shall next discuss the relation of the inclination of the ground to the direction of the curvature of the plant. First, the method of measurement will be explained. Suppose a plane made by three points a, b and c in the plant to be vertical (Fig. 11); then the line of projection of this plane on the surface of the slope will show the direction of the curvature of the plant. It was then ascertained by measuring whether this line of projection coincided with the direction of the steepest slope.[1]

In the sasa-community on which the test was made, the plane of the plant which coincided with the direction of the slope was found in the case of a large number of the plants. The plane of some plants, however, made a certain angle, and often a right angle, to the vertical plane through the direction of the slope; the converse case viz. that of the curvature of the plant against the inclination of the slope being rarely found. Quadrates on which sasa-communities grew were selected at random at many places varying in inclination. The following table (Tab. 3) shows the results of the measurements made of the plants in each one square metre quadrate. The inclination of the ground was measured with a clinometer. As will be seen in the table, the steeper the slope, the greater will be the percentage of the number of the plants which curve in the direction of the steepest slope. The results show that the direction of the curvature of the plant is influenced in the main by the degree of inclination of the slope.

Next, in order to determine the influence of the degree of inclination of the ground upon the curvature of the adult plant and of the young shoot respectively, the length of each internodium and the angle made by

[1] Viz the line which makes a right angle with the contour lines of the slope.

TABLE 3.

Relation of the inclination of the ground to the direction of the curvature of the plant.

Number of individual / Inclination of ground (s°)	n		o		a	
	Number	%	Number	%	Number	%
2.1	11	39	6	22	11	39
2.3	13	52	5	20	7	28
2.5	8	38	5	24	8	38
3.8	31	81	0	0	7	19
10.5	27	81	0	0	6	19
15.1	34	85	5	12	1	3
16.0	27	81	2	7	4	12
18.3	19	76	1	4	5	20
19.0	22	62	5	14	8	24
21.3	23	88	2	8	1	4
22.3	21	60	6	17	8	23
29.5	29	82	1	4	5	14
33.1	14	77	1	7	3	16
36.2	22	96	0	0	1	4
41.0	33	97	0	0	1	3
41.5	32	86	1	3	4	11

n, the culms which bend in the direction of the steepest slope; o, the culms which bend contrary to the direction of the slope; a, the culms which made a certain angle to the direction of the slope.

two internodia of the culm of the plants were compared with those of the young shoots, both of which were collected from two localities differing in inclination. The angle of inclination of the gentler plane was 2.5°, while that of the steeper slope was 46.5°. So far as adult plants are concerned, as is shown above in table 2, the length of the curved part of the plants collected from the locality with the smaller inclination is on an average 71.3 cm, while that of the plants collected from the locality with the greater inclination is about 84.3 cm. The same difference is seen also in the case of the young shoots. From these results, the conclusion will be arrived at that the length of the curvature portion is longer in the plants growing on a steep slope than that of those on a gentle slope. This difference seems to be attributable to the response of the culm to gravitation, though this explanation has not yet been confirmed experimentally.

SUMMARY.

1. A rhizome usually grows parallel to the surface of the soil, whether the ground is inclined or not. When a rhizome has attained a certain length, its growth ceases, and a new rhizome is either produced from the last node, or sprouts from a node of the culm under the ground.

2. Two types of curvature of the culm of a sasa plant as well as of its young shoot, can be distinguished, namely: one, in which the culm curves only once concavely under the ground, and the other, in which it curves twice, first concavely under the ground, and then convexly near the surface.

3. The second type in which the curve occurs twice, presumably arises from the first type, for almost all young shoots curve only once under the ground, while the second type is usually more common in the adult plants.

4. The result of the measurements of the curvature of the culms shows that the curvature generally begins at the 7-th or 8-th node, but that the culm becomes straight again at about the 20-th node. The degree of curvature between two adjoining nodes is on the average 172°.

5. The degree of convex curvature of an adult plant is greater than that of a young shoot, and the culm is inclined to bend to the ground more and more with age. This may, however, be due to the mechanical action of snow.

6. In a sasa-community, when the inclination of the ground increases, the number of plants which curve in the direction of the steepest slope also increases.

7. The length of the curved part of the culm is longer in plants growing on a steep slope than in those growing on a gentle slope.

8. Although the curvature of a culm is a peculiar property of a sasa plant, yet the direction of the curvature is affected by the inclination of the ground.

I here wish to express my hearty thanks to Prof. Dr. Y. YOSHII, Director of the Laboratory, under whose kind direction the present investigation has been carried out.

REPORT OF THE BIOLOGICAL SURVEY OF MUTSU BAY.
24. STOMATOPODA.[1]

BY

TAKU KOMAI.

Zoological Institute, Kyoto Imperial University.

(Received Aug. 30, 1932)

The collection comprises the following specimens which all belong to *Squilla oratoria* DE HAAN, the commonest stomatopod found in the Japanese waters:

No. 500. ♂1 (l. 180 mm.), ♀1 (l. 157 mm.). Loc.?

No. 501. ♂♂2 (l. 162, 172 mm.). Station 22, I. Off Kozima; Coll. Prof. S. HÔZAWA & Mr. S. TAKATUKI (July 20, 1926).

No. 502. ♂1(1.63 mm.). Station 55, II. Off Okuti; Coll. Prof. S. HÔZAWA & Mr. S. TAKATUKI (July 8, 1926).

No. 503. ♂1 (l. 155 mm.). Station 69, V. Off Ominato; Coll. Prof. S. HÔZAWA & Dr. S. KOKUBO (Aug. 11, 1926).

No. 504. ♀1 (l. 122.5 mm.). Station 2, I. Off Asamusi; Coll. Mr. T. MORIYAMA (June 20, 1925).

These have the characteristics of the 'Northern Form' of this species recorded in my previous paper (KOMAI, Mem. Coll. Sci. Kyoto Imp. Univ. B, III, p. 314). Mutsu Bay is apparently near the northern limit of the distribution of this species as well as of the whole group of the Stomatopoda in the Pacific. Although this species may be found in the coast of the southern districts of Hokkaidô, it is rather scanty there, and the bay is the northernmost locality where the stomatopod is fished in any abundance so as to form one of the objects of fisheries.

[1] Contributions from the Marine Biological Station, Asamushi, Aomori-Ken. No. 91.

REPORT OF THE BIOLOGICAL SURVEY OF MUTSU BAY.
25. CIRRIPEDIA.[1)]

BY

FUJIO HIRO.

Zoological Institute, Kyoto Imperial University.

(With four text-figures.)

(Received Aug. 30, 1932)

The present paper deals with the Cirripedia in Mutsu Bay, collected by Professor S. HÔZAWA, of the Tôhoku Imperial University, and by Professor S. HAREYAMA, of the Hiroshima Higher Normal School. The collections are represented by only the seven species which are all the forms prevalent in the Japanese waters. No cirriped, parasitic or commensal with other animals, is found. Thus, the cirripedian fauna of this bay seems to be rather poor. For all the species represented here, previous investigators such as PILSBRY and NILSSON-CANTELL have given full descriptions, so that it might be superfluous to reiterate here.

Finally, I wish to express my sincere thanks to Professor S. HÔZAWA for giving me the chance of examining the material.

KEY TO FAMILIES AND GENERA OF CIRRIPEDIA KNOWN TO OCCUR IN MUTSU BAY.

a. Pedunculate barnacles.

  b. Peduncle scaly; capitulum having 8 or more plates .... Family Scalpellidae.
  Genus *Mitella*.

  bb. Peduncle nude; capitulum having 5 large plates.............. ....Family Lepadidae.
  Genus *Lepas*.

aa. Sessile barnacles.

  b. Rostral compartment having alae overlapped by the adjacent rostrolateral compartment; walls composed of six compartments .... Family Chthamalidae.
  Genus *Chthamalus*.

  bb. Rostral compartment united with the adjacent rostrolateral compartment ....Family Balanidae.

1) Contributions from the Marine Biological Station, Asamushi, Aomori-Ken. No. 92.

c. Walls composed of six compartments......Genus *Balanus.*
cc. Walls porous, composed of four compartments ....
.... Genus *Tetraclita.*

Family **Scalpellidae** PILSBRY.

Genus MITELLA OKEN.

1. **Mitella mitella** (LINNÉ).

Syn. PILSBRY, 1907; NILSSON-CANTELL, 1921.

This species is represented by numerous specimens which carry some individuals of *Chthamalus challengeri* on the capitulum. The largest specimen measures 28 mm. in breadth and 68 mm. in length.

Locality: Futago-jima. Coll. Prof. S. HÔZAWA; Prof. S. HAREYAMA.

Distribution: Widely distributed from Japan to the Malayan waters.

Family **Lepadidae** DARWIN.

Genus LEPAS LINNÉ.

2. **Lepas anatifera** LINNÉ.

Syn. NILSSON-CANTELL, 1921.

The specimens, which are attached to a floating bamboo-stem, correspond with the typical form of this species; the surface of the scuta is marked with a diagonal broken line of hellebore green, while on the surface of the terga there is no trace of it.

Locality: Off Jizôson near the Asamushi Marine Biological Station. Coll. Mr. T. MORIYAMA.

Distribution: Pelagic, cosmopolitan.

Family **Chthamalidae** DRAWIN.

Genus CHTHAMALUS RANZANI.

3. **Chthamalus challengeri** HOEK.

(Text-figs. 1, 2.)

Chthamalus challengeri HOEK, 1883, and other later authors.
Chthamalus stellatus WELTNER, 1897 (in part); KRÜGER, 1911.
Chthamalus challengeri nipponensis PILSBRY, 1916.

Chthamalus challengeri occurs in the Japanese waters in crowd on *Mitella mitella* and *Tetraclita squamosa japonica*, as well as on rocks in the littoral zone. It is very difficult to make a distinction between *C. challengeri* and *C. stellatus* (POLI, 1795) by the mere external appearance,

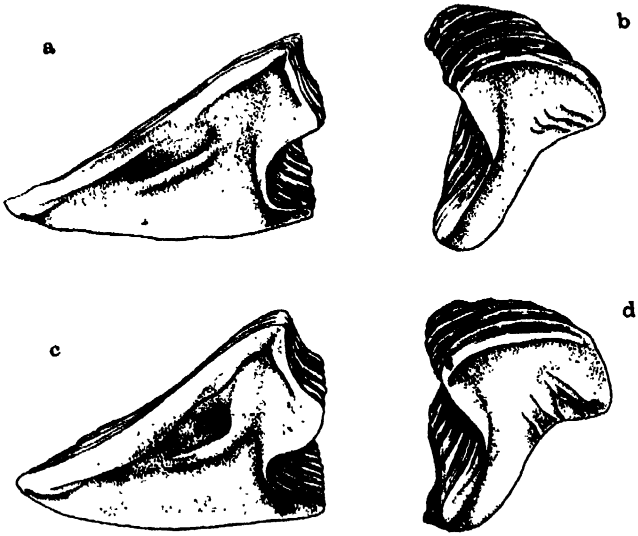
and they have been often confused, as it was done by WELTNER (1897) and KRÜGER (1911).

The difference may be found in that *C. challengeri* has a well-developed adductor ridge on the scutum, while it is only feebly represented in *C. stellatus*. The latter species is widely distributed in the Mediterranean and the Atlantic; it is also known in the Malay Archipelago, but not yet in the Japanese waters.

In the external appearance of this species there are developmental and individual variations, as is also the case with *C. stellatus*. In fact the numerous specimens which were obtained in Mutsu Bay, differ to some extent from the southern forms, e. g., those from Seto or Misaki, in both the external and internal features. In most of the southern specimens, the walls show many radiating ribs and a punctate appearance, as in a form of *C. stellatus* (Cf. PILSBRY, 1916: Pl. 71, Fig. 3.); the walls also are rather fragil and the orifice is much larger than that of the northern form. The latter form, *viz.*, the specimen collected in Mutsu Bay, has almost smooth walls, and when it is found in crowd, it becomes cylindrical, and up to about 10 mm. in height, much like the tubular form of *C. stellatus* (Cf. PILSBRY, 1916: Pl. 71, Fig. 2 b.).

In the opercular valves also, there is a little difference between the northern and southern forms. In small specimens in this collection the opercular valves bear similarity to HOEK's figure of the typical form of this species (Cf. HOEK, 1883: Pl. XIII, Figs. 37, 38.). In all the depressed and tubular forms, the scutum is rather elongate laterally and the tergum has a narrow spur; these valves correspond perfectly with PILSBRY's figure of those of the specimens from Matsushima and from Ayukawa which are also located in the northern Japan (Cf. PILSBRY, 1916: Pl. 72, Figs. 1, 1a, 2, 2a.). (Text-fig. 1, a. b.) In the specimens from Seto or Misaki, on the other hand, the scutum is rather wide and has a very strong articular ridge, and the tergum has a rather wide spur; the valves resemble closely the same author's figure of the same of the specimens from Yokohama which is near Misaki (Cf. PILSBRY, 1916: Pl. 72, Figs. 4, 4a.). (Text-fig. 1, c, d.)

In short, the differences found in these valves seem to be only local variation. However, the opercular valves, which are figured by NILSSON-CANTELL (1925) from a specimen from the Bonin Islands, show some resemblance to those of the northern form, in spite of the Islands being located far in the southern sea. Moreover the opercular valves of the specimen from Sagami Bay, which is described and figured by KRÜGER



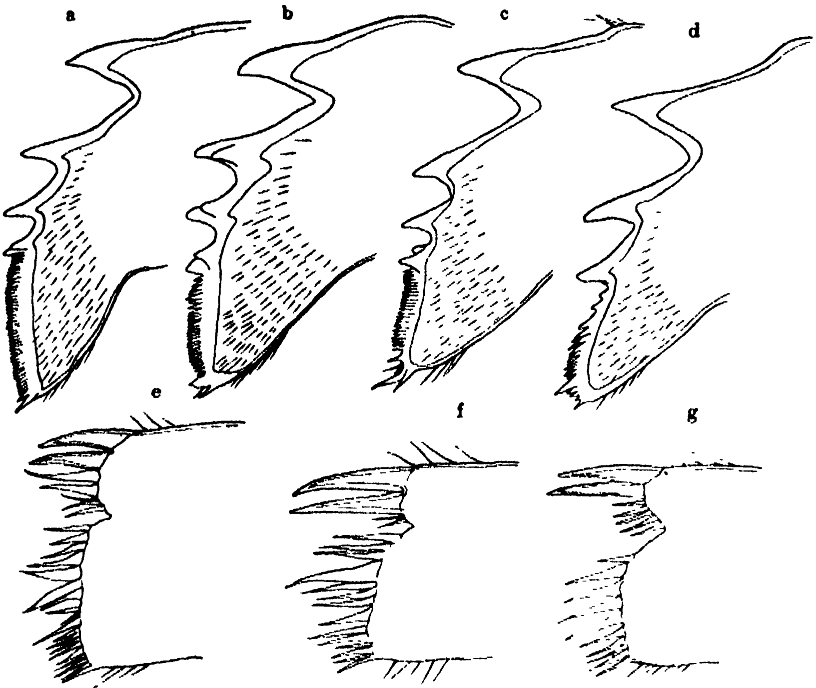
Text-fig. 1. *Chthamalus challengeri* HOEK.
a, Scutum, *b*, tergum, of a specimen from Mutsu Bay. ×17.
c, Scutum, *d*, tergum, of a specimen from Seto. ×17.

(1911) to be of *C. stellatus*, show a resemblance to those of the northern form. The disagreement of the opercular valves to be noted in these reports seems to be due to the variation in the shape of the external walls according to the state of environment.

As regards the mouth-parts, PILSBRY (1916) points out the mandible to bear a characteristic of the racial significance; he says: "The mandible is of the *stellatus* form, but the three points at the lower extremity are much more strongly developed and the finely pectinated space above them is shorter." As a matter of fact, in most of the specimens from Seto the mandible has a shorter pectinated space, as PILSBRY says, and the first maxilla bears deeper notch than found in *C. stellatus*. But the mouth-parts of the specimens from Mutsu Bay agree more closely with those of *C. stellatus*, while among the specimens from Seto and Misaki there is often the state intermediate between these two forms. (Text-fig. 2, a — g.)

It is probably certain that the specimen from Hakodate recorded by WELTNER (1897) as *C. stellatus is C. challengeri*.

Locality: Futago-jima. Coll. Prof. S. HÔZAWA; Prof. S. HAREYAMA.
Distribution: Japanese and Malayan waters.



Text-fig. 2. *Chthamalus challengeri* HOEK.
a — *d*, Mandible of specimens from Mutsu Bay (*a*, *b*), from Misaki (*c*), from Seto (*d*).
e — *g*, Maxilla I of specimens from Mutsu Bay (*e*, *f*), from Seto (*g*).

Family **Balanidae** GRAY.

Genus BALANUS DA COSTA.

4. **Balanus tintinnabulum rosa** PILSBRY.

(Text-fig. 3)

Balanus tintinnabulum rosa PILSBRY, 1916; BROCH, 1931; NILSSON-CANTELL, 1931, 1932.

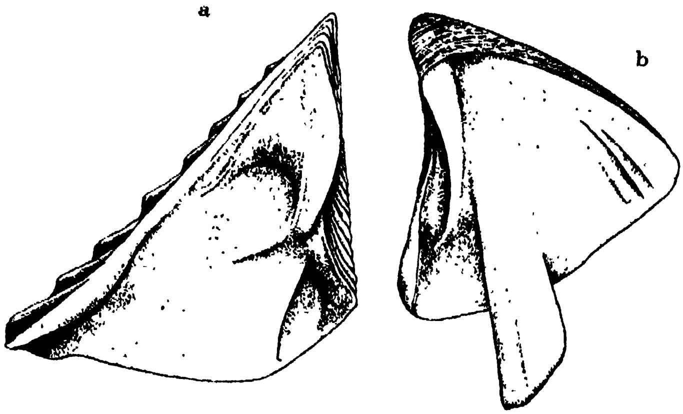
The collection includes several specimens of this species, attached to the rocks and to the shell of *Mytilus crassitesta* LISCHKE. These correspond well with PILSBRY's original statement "The barnacle is conic or subcylindric, with a rather large, broadly and acutely ovate aperture; roseate (between pomegranate-purple and Indian-lake of RIDGWAY's Color Standards), the parietes of rostrum and lateral compartments paler than the carina, the radii a deeper shade of the same color. Sheath a duller shade of the external color." Although the beautiful color is characteristic of this subspecies, there are white forms in small number mingled with

the reseate forms; the former is entirely white in the young stage, and the adult specimen bears the white parietes, though the radii and sheath are tinged with very pale roseate hue.

The opercular valves (Text-fig. 3, a, b.) and mouth-parts agree with the description of NILSSON-CANTELL (1932).

Locality: Futago-jima. Coll. Prof. S. HÔZAWA.

Distribution: Japan.



Text-fig. 3. *Balanus tintinnabulum rosa* PILSBRY.
a, Scutum, *b*, tergum. ×6.

5. Balanus rostratus eurostratus BROCH.

(Text-fig. 4.)

Syn. PILSBRY, 1916; HIRO, 1932.

The specimens from Mutsu Bay agree perfectly with *Balanus rostratus* HOEK, forma *eurostratus* (BROCH, 1922). There are numerous small and large specimens up to 49 mm. in carino-rostral length and 80 mm. in height. In the largest specimen, which is dry and bears a very corroded surface, the orifice is almost as large as the base and the rostrum is furnished with 18 parietal tubes, like those of *B. rostratus apertus* PILSBRY, 1911 from Bering Sea, though the parietal tubes are with many transverse septa which extend quite to the base. The number of parietal tubes is individually different as mentioned by NILSSON-CANTELL (1932) for the examples of *B. rostratus spiniferus* from Kobe; a specimen measuring about 28 mm. in carino-rostral length in this collection has 14 tubes in the rostrum.

Locality: Mutsu Bay. Coll. Prof. S. HÔZAWA; Prof. S. HAREYAMA.
Distribution: Japan.

Text-fig 4 *Balanus rostratus eurostratus* BROCH. × 1.

6. Balanus trigonus DARWIN.

Balanus trigonus DARWIN, 1854, and other later authors

Locality: Mutsu Bay. Coll. Prof. S. HÔZAWA.
Distribution: Pacific, Indian and Southern Atlantic Ocean.

Genus TETRACLITA SCHUMACHER.

7. Tetraclita squamosa japonica PILSBRY.

Tetraclita porosa var *nigrescens* KRÜGER, 1911.
Tetraclita porosa PILSBRY, 1911,
Tetraclita squamosa japonica PILSBRY, 1916.
Tetraclita porosa japonica NILSSON-CANTELL, 1927, 1931, 1932.

This subspecies is the prevalent form of the species in the Japanese waters.

Locality: Futago-jima. Coll. Prof. S. HÔZAWA.
Distribution: Japan.

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REPORT OF THE BIOLOGICAL SURVEY OF MUTSU BAY.
24. THE PELAGIC CILIATA, SUBORDER TINTINNOINEA[1].

By

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(With 26 text-figures)

(Received October 3, 1932)

INTRODUCTION

The present work is based upon the materials obtained by myself in surface collections and vertical hauls during August, 1929–September, 1930 at numerous stations scattered throughout Mutsu Bay, and upon the plankton collected with a surface tow net twice a month during 1927–1931 and preserved in the Asamushi Marine Biological Station of the Tôhoku Imperial University.

In this paper I have recorded 34 species belonging to 12 genera and 8 families according to KOFOID and CAMPBELL's systematic arrangement of the Tintinnoinea (1929). Of these, 7 seem to be new to science.

It is my pleasure and duty to offer my sincere acknowledgment to Professor Dr. C. A. KOFOID and Dr. A. S. CAMPBELL, of the Zoological Department of the University of California, for their kind advice and help in identification of the species and in the accomplishment of this investigation. In collection and examination of the materials I am indebted to Dr. S. KOKUBO and Mr. T. TAMURA, of the Asamushi Marine Biological Station.

SYSTEMATIC PART

Class CILIATA.

Order HETEROTRICHIDA.

Suborder **Tintinnoinea**.

Key to genera.

1. Lorica consisting of a bowl with or without an aboral horn; wall

[1] Contributions from the Marine Biological Station, Asamushi, Aomori-Ken. No. 93.

composed of a fine primary structure and an agglomerated material. Genus *Tintinnopsis*.

2. Lorica with a very low, hyaline collar and a coarsely constituted bowl. Genus *Stenosemella*.
3. Lorica consisting of a higher, hyaline, subcylindrical collar with spiral turns and a coarser bowl with an alveolar pattern or agglomerated particles. Genus *Codonellopsis*.
4. Lorica usually bell-shaped, with a flaring callar divided by a nuchal constriction from a bowl; wall bilamellate, with an irregularly polygonal reticulation. Genus *Cyttarocylis*.
5. Lorica chalice-shaped; wall composed of separated lamellae with fine, subuniform, hexagons. Genus *Parafavella*.
6. Lorica bell-shaped, having two elevated bands; wall weakly bilaminated; surface with minute irregular polygons or fine plications. Genus *Ptychocylis*.
7. Lorica with double collars, inner one a little higher than the outer denticulated with short triangular teeth; wall bilamellate, usually hyaline, but not structureless. Genus *Acanthostomella*.
8. Lorica elongated chalice-shaped; aboral end drawn out into a simple caudal lance; wall generally hyaline, composed of almost separated laminae fused partially in the aboral end. Genus *Parundella*.
9. Lorica vase- or cup-shaped, with a fairly developed inner collar by the reason of the thickened wall in the suboral region; wall trilamellate, translucent. Genus *Proplectella*.
10. Lorica consisting of a low funnel-shaped collar and an elongated bowl with a few ridges or lines; oral rim entire and circular; wall translucent. Genus *Amphorella*.
11. Lorica subcylindrical, open at the both ends; wall typically hyaline. Genus *Tintinnus*.
12. Lorica elongate, tubular; collar an inverted, truncate, conical cone; shaft subcylindrical, with some fins at the aboral region; aboral end usually open; wall almost hyaline. Genus *Salpingella*.

Family **Codonellidae.**

Genus TINTINNOPSIS STEIN, 1867.

1. **Tintinnopsis beroidea** STEIN.

Tintinnopsis beroidea STEIN, *1867; KOFOID and CAMPBELL, 1929, p. 28, fig. 26; HADA,

*Indicates literature which I have not examined.

1932 b, p. 41, fig. 2.

Length, 61(50–74)μ; oral diameter, 35(31–40)μ.
Occurs in February-April and September; common.

2. Tintinnopsis urnula MEUNIER.

Tintinnopsis urnula MEUNIER, 1910, p. 145, pl. 13, figs. 21–25; KOFOID and CAMPBELL, 1929, p. 50, fig. 20; HADA, 1932 b, p. 42. fig. 3.

Length, 60 μ; oral diameter, 40 μ.
Occurs in March and April; very rare.

3. Tintinnopsis tubulosoides MEUNIER.

Tintinnopsis tubulosoides MEUNIER, 1910, p. 139, pl. 12, figs. 10, 11; KOFOID and CAMPBELL, 1929, p. 49, fig. 74; HADA, 1932 b, p. 43, fig. 5.

Length, 94(83–104)μ; oral diameter, 36(34–40)μ.
Occurs in April; rare.

4. Tintinnopsis tenuis, n. sp.

Text-figure 1.

Lorica elongated capsular, 2.0–2.5 oral diameters in length; oral rim usually entire; bowl cylindrical; aboral end generally hemispherical; wall thin, subuniform, 0.03 of the oral diameter in thickness, showing a slight spiral structure in the suboral part, with fine and sparse agglomerations.

Length, 60(54–64)μ; oral diameter, 27(25–29)μ.

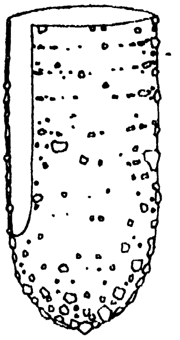
Occurs in May-October; rare.

Differs from *Tintinnopsis acuminata* DADAY and *Tps. beroidea* STEIN in the rounded aboral end and in the presence of the faint spiral structure, from *Tps. karajacensis* BRANDT and *Tps. rotundata* JÖRGENSEN in dimensions and in a sparse agglomerated material, and from *Tps. minuta* WAILES in being larger and in more slender proportions.

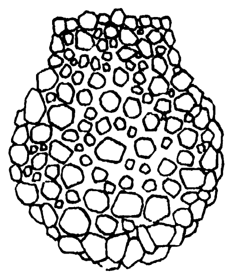
5. Tintinnopsis conglobata, n. sp.

Text-figure 2.

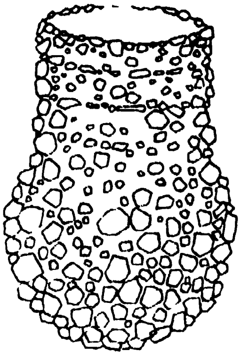
Lorica ovoidal, 2.3 oral diameters in length, consisting of a short subcylindrical collar and an ovate bowl, widest near the middle, its greatest transdiameter 1.8 of the oral diameter; oral rim ragged: shoulder gradually sloping; aboral end rounded; wall rather coarse and thick, without a spiral structure.



Text-fig. 1. *Tintinnopsis tenuis*, n. sp. ×750.



Text fig. 2. *Tintinnopsis conglobata*, n. sp. ×750.



Text-fig. 3. *Tintinnopsis lohmanni* LAACKMANN. ×750.

Length, 46(44–48)μ; oral diameter, 20 μ.

Occurs in February-April; rare.

Differs from *Tintinnopsis compressa* DADAY in the presence of the marked subcylindrical nuchal part and from *Tps. nucula* (FOL) in the widened bowl, in the hemispherical aboral end and in the less shouldered collar.

6. Tintinnopsis lohmanni LAACKMANN.

Text-figure 3.

Tintinnopsis lohmanni LAACKMANN, 1906, p. 20, pl. 1, figs. 10, 11, pl. 2, fig. 23.
Tintinnopsis sp. BRANDT, 1906, pl. 17, figs. 1, 3; 1907, p. 180.
Tintinnopsis tubulosa, var. *lohmanni*, JÖRGENSEN, 1927, p. 7.
Tintinnopsis subacuta (part), KOFOID and CAMPBELL, 1929, p. 47.

Lorica stout flask-shaped, 2 oral diameters in length, cylindrical anteriorly, enlarged aborally; oral rim more or less irregular, no oral flare; tubular part 0.40–0.43 of the total length in length, provided with 2 or 3 spiral turns; aboral region subspherical or broadly conical, 1.14–1.23 oral diameters in transdiameter at the posterior 0.3 of the total length; wall agglomerated rather coarsely, 0.08 oral diameters in thickness.

Length, 60 μ; oral diameter, 30 μ.

Occurs in August; rare.

Differs from *Tintinnopsis compressa* DADAY in contour being composed of the oral cylindrical part and the expanding aboral region, from *Tps. subacuta* JÖRGENSEN in the shortened anterior tubular part and in the shape of the aboral end, and from *Tps. turgida* KOFOID and CAMPBELL in the short suboral region and in the presence of the spiral structure in the same portion.

7. Tintinnopsis directa, n. sp.

Text-figure 4.

Tintinnopsis sp. (*T. campanula* var.?) OKAMURA, 1907, p. 139, pl. 6, fig. 64.
Tintinnopsis patula (part), KOFOID and CAMPBELL, 1929, p. 43.

Lorica tall campanulate, 1.6–2.2 oral diameters in length; oral rim irregular, flaring (60°–92°); suboral region somewhat tapering, conical (5°–10°), laid up with about 6 spiral turns, narrowest at the basal portion of the subcylindrical part, its smallest transdiameter 0.68–0.82 of the oral diameter; posterior region subspherical, with a rounded aboral end, 0.80–0.95 oral diameters in transdiameter; wall rather coarse in the posterior part, about 0.035 diameters in thickness at the thickest portion of the aboral region.

Length, 88(72–100)μ; oral diameter, 42(40–45)μ; greatest transdiameter, 38(34–40)μ.

Type locality, off Tosa, Japan.

Occurs in July-October; common.

Differs from *Tintinnopsis dadayi* KOFOID in the elongated lorica and in the coarse surface, from *Tps. everta* KOFOID and CAMPBELL in having a distinct aboral enlargement, from *Tps. pallida* BRANDT in the presence of the more differentiated aboral part, and from *Tps. turgida* KOFOID and CAMPBELL in possession of a flare of the oral rim.

8. Tintinnopsis bütschlii DADAY.

Text-figure 5.

Tintinnopsis Bütschlii DADAY, 1887, p. 556, pl. 20, figs 4, 5; KOFOID and CAMPBELL, 1929, p 29, fig. 85.
Tintinnopsis campanula var b *bütschlii* (part), BRANDT, 1907, p. 151.

Lorica campanulate, consisting of a broadly expanding and everated oral region and a convex conical bowl, its length 0.94–1.05 oral diameter; oral rim roughened, conical (about 130°); bowl narrowest at the upper third of the lorica, its least transdiameter 0.41–0.45 of the oral diameter, dilated posteriorly a little (7°–9°), 0.43–0.48 of the oral diameter in greatest transdiameter at the posterior 0.25 of the total length; aboral end hemispherical; wall 0.023–0.027 oral diameters in thickness, with a trace of a spiral structure in the suboral nuchal region.

Length, 88(84–92)μ; oral diameter, 88(80–92)μ; greatest transdiameter of the bowl, 40(38–43)μ.

Occurs in August-October; common.

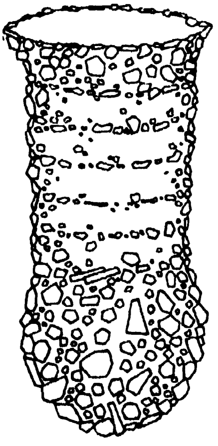
Differs from *Tintinnopsis cyathus* DADAY and *Tps. everta* KOFOID and CAMPBELL in the more spreading oral flare and from *Tps. mortensenii* SCHMIDT in the shape of the oral flare which is not so much everted in this species as in the last.

9. Tintinnopsis karajacensis BRANDT.

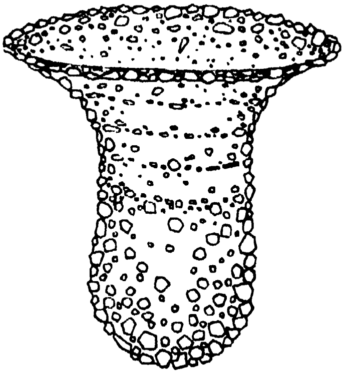
Text-figure 6.

Tintinnopsis karajacensis BRANDT, 1896, p. 57, pl. 3, fig. 5; 1906 (part), 19, figs. 5, 10, 12, pl. 26, fig. 3; 1907, p. 162; LAACKMANN, 1906, p. 21, pl. 1, figs. 12–14; JÖRGENSEN, 1927, pp. 5, 7; KOFOID and CAMPBELL, 1929, p. 37, fig. 38.

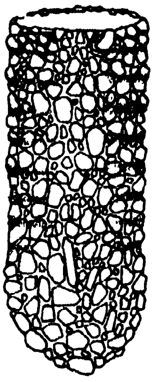
Lorica cylindrical, 2.0–2.7 oral diameters in length, oral rim ragged; aboral end rounded or disfigured as the result of irregularly agglomerated particles; wall coarse, having several slight spiral turns in the anterior half.



Text-fig. 4. *Tintinnopsis directa*, n. sp. ×650.



Text-fig. 5 *Tintinnopsis bütschlii* DADAY ×550.



Text-fig. 6. *Tintinnopsis karajacensis* BRANDT ×300.

Length, 111–172 μ; oral diameter, 55–64 μ.

Occurs in June and July; rare.

Differs from *Tintinnopsis cochleata* (BRANDT) in less extensive spiral organization and in roughened agglomeration, from *Tps. lobiancoi* DADAY in the shorter lorica, and from *Tps. rotundata* JÖRGENSEN in more slender proportions and in the shape of the aboral end.

10. Tintinnopsis lobiancoi DADAY.

Text-figure 7.

Tintinnopsis Lobiancoi DADAY, 1887, pp. 545, 553, pl. 19, fig. 27; CLEVE, 1900 a, p. 17,

fig. 4; 1900 b, p. 18; BRANDT, 1906, pl. 19, fig. 3, pl. 24, fig. 16, pl. 26, figs. 7, 8; 1907, p. 160; OKAMURA. 1907, p. 137, pl. 6, fig. 56; ENTZ, Jr. (part), 1909, pl. 9, fig. 2, pl. 12, fig. 4, pl. 21, fig. 6; MERKLE, 1909, p. 153, pl. 2, figs. 13, 24; MEUNIER, 1910, p. 138, pl. 12, figs. 5-9; JÖRGENSEN, 1927, pp. 5, 7; KOFOID and CAMPBELL, 1929, p. 38, fig. 95.

Tintinnopsis radix forma *subrotundata* LAACKMANN, 1913, p. 23, pl. 2, fig 32.

Tintinnopsis radix forma *curta-subrotundata* LAACKMANN, 1913, p. 23, pl. 2, fig. 34.

Lorica elongate, tubular, usually straight, 4.5 oral diameters in length; oral rim ragged; aboral end rounded or shaped somewhat irregularly; wall agglomerated roughly, but comparatively thin, 0.04 of the oral diameter in thickness, without a spiral structure.

Length, 151 μ; oral diameter, 34 μ.

Occurs in September; very rare.

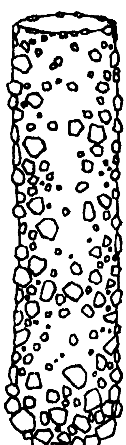
Differs from *Tintinnopsis karajacensis* BRANDT in the longer lorica and in slender proportions and from *Tps. cochleata* (BRANDT) in lack of the spiral structure.

11. Tintinnopsis tocantinensis KOFOID and CAMPBELL.

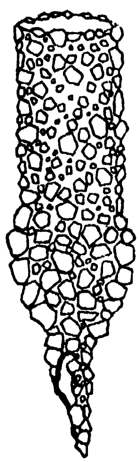
Text-figure 8.

Tintinnopsis aperta var. a BRANDT, 1906, pl. 25, figs. 2, 7; 1907 pp. 129, 177.

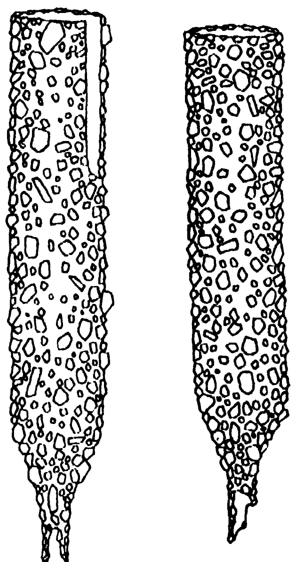
Tintinnopsis tocantinensis KOFOID and CAMPBELL, 1929, p. 48, fig. 46.



Text-fig. 7. *Tintinnopsis lobiancoi* DADAY ×400.



Text-fig 8. *Tintinnopsis tocantinensis* KOFOID and CAMPBELL ×600.



Text-fig. 9. *Tintinnopsis kofoidi* HADA ×350.

Lorica elongated, 4.7 oral diameters in length, cylindrical anteriorly, expanding posteriorly, tapering distally into a stout aboral horn; dilated part not spiraled, 1.2 of the oral diameter in transdiameter; aboral horn conical (35°), obliquely or irregularly open at the tip; wall thick and coarse.

Length, 103 μ; oral diameter, 22 μ.

Occurs in September; very rare.

Differs from *Tintinnopsis aperta* BRANDT in the absence of the spiral structure at the enlarged region and in having the stout aboral horn.

12. Tintinnopsis kofoidi HADA.

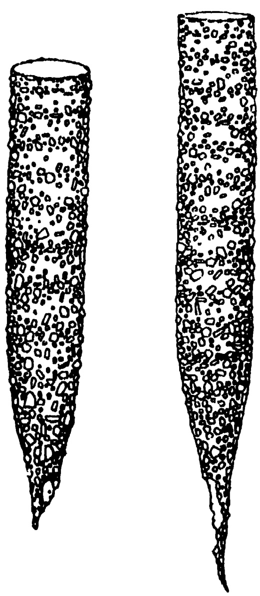
Text-figure 9.

Tintinnopsis kofoidi HADA, 1932 a, p. 210, figs. 2, 3; 1932 b, p. 44, fig. 6.

Occurs in July; rare.

13. Tintinnopsis radix (IMHOF) BRANDT.

Text-figure 10.



Text-fig. 10. *Tintinnopsis radix* (IMHOF) ×250.

Codonella radix IMHOF, 1886, p. 103.

Tintinnopsis Davidoffii DADAY, 1887, p. 552, pl. 19, fig. 23.

Tintinnopsis Davidoffii var. *cylindrica* (part), DADAY, 1887, p. 553, pl. 19, fig. 25.

Tintinnopsis Davidoffii var. *longicauda*, DADAY, 1887, pp. 545, 553, pl. 19, fig. 26.

Tintinnopsis curvicauda DADAY, 1887, p. 554, pl. 19, fig. 33

Tintinnopsis fracta BRANDT, 1906 pl. 23, figs. 1, 3-5, 9-13, pl. 31, fig. 8; 1907, p. 174; OKAMURA, 1907, p. 137, pl. 6, fig. 57.

Tintinnopsis radix, BRANDT, 1907, p. 20; LAACKMANN, 1913, p. 17, pl. 2, figs. 17-20, 27-28; KOFOID and CAMPBELL, 1929, p. 45, fig. 93.

Tintinnopsis radix forma *typica* LAACKMANN, 1913, p. 22.

Tintinnopsis radix forma *curta* LAACKMANN, 1913, p. 23, pl. 2, figs. 21-24, 26.

Tintinnopsis radix forma *cylindrica*, LAACKMANN, 1913, p. 23, pl. 2, figs. 25, 29-31.

Lorica elongate, slender, tubular, 6.0-9.5 oral diameters in length; oral rim generally

entire, sometimes irregular; bowl long, cylindrical; aboral region tapering gradually into an aboral horn, inverted conical (41°–21°); aboral horn usually more or less curved, with an irregularly formed aboral opening typically set laterally as gouged, leaving its tip or cutting off it; wall thin and fragile, 0.03 of the oral diameter in thickness, with a slight spiral structure.

Length, 337(260–416)μ; oral diameter, 43(40–45)μ.

Occurs in August-October; common.

Differs from *Tintinnopsis kofoidi* HADA in the fragile construction of the lorica, in less contraction at the aboral region, and in the shape of the lateral opening in the aboral horn.

Family **Codonellopsidae.**

Genus STENOSEMELLA JÖRGENSEN, 1924.

14. **Stenosemella nivalis** (MEUNIER) KOFOID and CAMPBELL.

Text-figure 11.

Codonella ventricosa, ENTZ, Sr., 1884, p. 413, pl. 24, fig. 24.
Tintinnopsis ventricosa, DADAY, 1887, pp. 546, 559, pl. 20, figs. 19, 20.
Tintinnopsis nucula (part), LAACKMANN, 1906, p. 19, pl. 1, fig. 4, pl. 3, figs 48–50; CAMPBELL, 1926, pp. 179–236, pl. 12–15, text-figs A-G.
Tintinnopsis nivalis MEUNIER, 1910, p. 143, pl. 13, figs. 26, 27.
Stenosemella nucula, JÖRGENSEN, 1927, p. 8, fig. 7.
Stenosemella nivalis, KOFOID and CAMPBELL, 1929, p. 69, fig. 136.

Lorica ovoidal, 1.8–2.2 oral diameters in length; collar somewhat concave conical, 0.08–0.10 of the total length in height; bowl widest a little upper the middle of the lorica, 1.9 oral diameters in transdiameter; aboral region subacute; wall of the collar thin and hyaline, about 0.05 of the oral diameter in thickness, sometimes with a few foreign particles, wall of the bowl with a rather coarse agglomerated material.

Length, 43(40–44)μ; oral diameter, 21(20–22)μ; transdiameter of the bowl, 39(36–41)μ.

Occurs throughout the year; rare.

Differs from *Stenosemella pacifica* KOFOID and CAMPBELL in lack of fenestrae at the base of the collar and from *S. ventricosa* (CLAPARÈDE LACHMANN) in dimensions.

Genus CODONELLOPSIS JÖRGENSEN, 1924.

15. **Codonellopsis pusilla** (CLEVE) KOFOID and CAMPBELL.

Text-figure 12.

Codonella pusilla CLEVE, * 1900; BRANDT, 1907, p. 120.

Codonellopsis pusilla, KOFOID and CAMPBELL, 1929. p. 87. fig. 146.

Lorica stout fusiform, 2.6 oral diameters in length; collar subcylindrical in the anterior 0.6 of its length and posteriorly conical (55°), then gradually changing into an ovoidal bowl, with about 13 spiral turns extending towards the bowl; bowl ovate, 1.8 of the oral diameter in transdiameter; aboral region an inverted cone of 125°, with a blunt aboral end; wall nearly uniform in thickness throughout the lorica, composed of an alveolar structure and very few agglomerated particles.

Length, 56 μ; oral diamer, 21 μ; transdiameter, 37 μ.

Occurs in July; very rare.

Differs from *Codonellopsis contracta* KOFOID and CAMPBELL in lack of fenestration of the basal portion of the collar and in the subacute aboral end.

16. Codonellopsis contracta KOFOID and CAMPBELL.

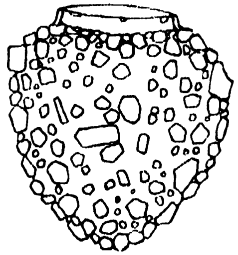
Text-figure 13.

Codonellopsis contracta KOFOID and CAMPBELL, 1929, p. 78, fig. 147.

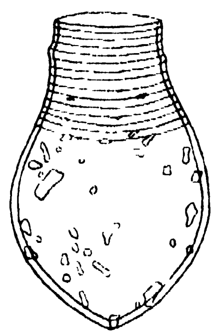
Lorica ovate, 2.1 oral diameters in length; collar 0.31–0.35 of the total length in length, usually with 6 spiral turns, concave conical (30°–40°), provided with a few, transversely elliptical fenestrae in its basal part; bowl globose, 1.7 of the oral diameter in thickness, with primary and secondary structures and few agglomerated particles.

Length, 43(40–45) μ; oral diameter, 22(20–23) μ, transdiameter, 36(35–37) μ.

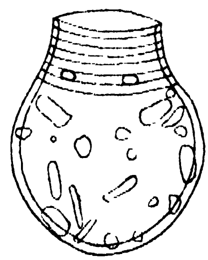
Occurs in July; rare.



Text-fig. 11. *Stenosemella nivalis* (MEUNIER) ×750.



Text-fig. 12. *Codonellopsis pusilla* (CLEVE) ×750.



Text-fig. 13 *Codonellopsis contracta* KOFOID and CAMPBELL ×750.

Differs from *Codonellopsis frigida* HADA in having fenestrae and in the smoothly sloping shoulder and from *C. pusilla* (CLEVE) in the presence

of fenestration at the lower part of the collar, in the comparatively small number of spiral turns, and in its hemispherical aboral end.

17. **Codonellopsis limosa**, n. sp.

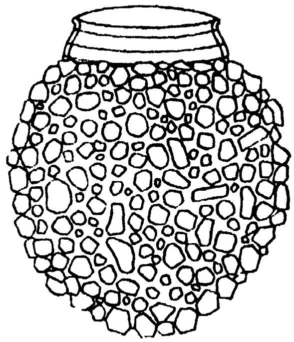
Text-figure 14.

Lorica ovoidal, 2.4 oral diameters in length; collar short with a little oral eversion, 0.13 of the total length in length, convex conical (30°), figured with a few spiral turns; bowl ovate, widest near the middle of the lorica, 1.8 oral diameters in transdiameter; aboral region hemispherical; wall agglomerated neatly with small particles.

Length, 82 μ; oral diameter, 33 μ; greatest transdiameter, 63 μ.

Occurs in January; very rare.

Differs from all other allied species of *Codonellopsis morchella* (CLEVE) in the short collar and in fewer spiral turns.

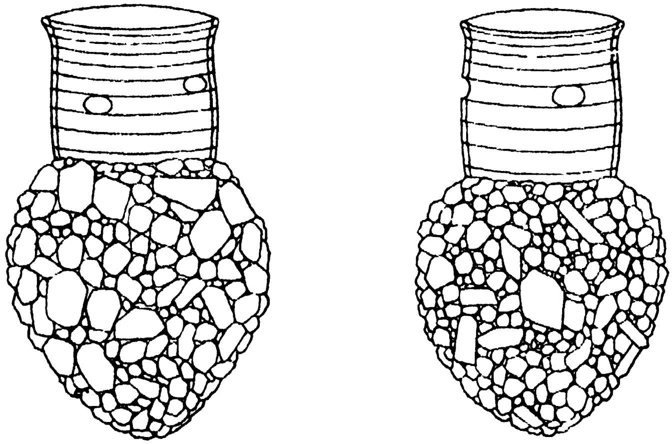


Text-fig. 14. *Codonellopsis limosa*, n. sp. ×550.

18. **Codonellopsis orientalis**, n. sp.

Text-figure 15.

Lorica consisting of a subcylindrical collar and an ovate bowl, 2.5–2.7 oral diameters in length; oral rim flaring; collar 0.30–0.42 of the total



Text-fig. 15. *Codonellopsis orientalis*, n. sp. ×650.

length in length, constricted slightly in the suboral part and bulging out in the middle, with 8–11 spiral turns increasing in width towards the bowl, a few fenestrae appearing in the dilated region; bowl widest a little above the middle, contracting aborally; aboral region broadly convex conical (85°–105°); aboral end blunt; wall coarsely agglomerated.

Length, 90(84–92)μ; oral diameter, 34(32–34)μ; greatest transdiameter, 50 μ.

Occurs in September-January; common.

Differs from *Codonellopsis americana* KOFOID and CAMPBELL, *C. erythräensis* (BRANDT), and *C. indica* KOFOID and CAMPBELL in the conical aboral region and from *C. morchella* (CLEVE) in an everted oral margin and in the shape of the collar.

Family **Cyttarocylidae.**

Genus CYTTAROCYLIS FOL, 1881.

19. **Cyttarocylis magna** BRANDT.

Text-figure 16.

Cyttarocylis cassis var. c *magna* BRANDT, 1906, pl. 34, fig. 3, pl. 35, fig. 3; 1907, pp. 189, 196; LAACKMANN, 1909, p. 447.

Cyttarocylis magna, KOFOID and CAMPBELL, 1929, p. 114, fig. 222; KOFOID, 1930, fig. 29 (No. 222).

Lorica consisting of a low funnel-shaped collar and a tall inverted conical bowl of angles changing from 15° in the anterior half to 45° in the aboral region, its length 1.8–2.2 oral diameters; oral rim irregularly dentate; collar convex conical (42°), 0.9 oral diameters in transdiameter, 0.06 of the total length in length; aboral horn short, 0.05 of the total length in length, shaped irregularly; wall with a comparatively larger polygonal reticulation.

Length, 300 μ; oral diameter, 150 μ.

Occurs in November; rare.

Differs from *Cyttarocylis acutiformis* KOFOID and CAMPBELL in the coarsely reticulated wall and in having the marked aboral horn and from *C. cassis* (HAECKEL) in the tall tapering bowl.

Genus PARAFAVELLA KOFOID and CAMPBELL, 1929.

20. **Parafavella denticulata** (EHRENBERG) KOFOID and CAMPBELL.

Tintinnus denticulatus EHRENBERG, *1840.

Parafavella denticulata, KOFOID and CAMPBELL, 1929, p. 163, fig. 310; HADA, 1932 b, p. 50, fig. 15.

Length, 225(200–235)μ; oral diameter, 63(62–64)μ.
Occurs in January-March; common.

21. Parafavella gigantea (BRANDT) KOFOID and CAMPBELL.

Cyttarocylis gigantea (part) BRANDT, 1896, p. 63, pl. 3, figs. 21, 24.
Parafavella gigantea, KOFOID and CAMPBELL, 1929, p. 165, fig 311; HADA, 1932 b, p. 51, fig. 16.

Length, 380(337–486)μ; oral diameter, 65(63–69)μ.
Occurs in January-May; common.

22. Parafavella pacifica HADA.

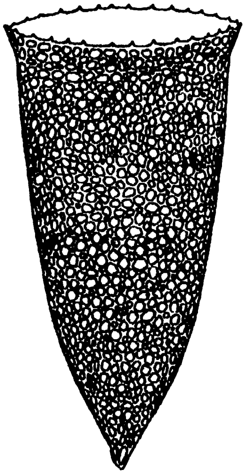
Parafavella pacifica HADA, 1932 b, p. 49, fig 13

Length, 135(120–154)μ; oral diameter, 47(45–52)μ.
Occurs in June-September; common.

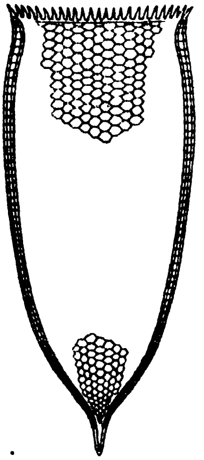
23. Parafavella faceta, n. sp.

Text-figure 17

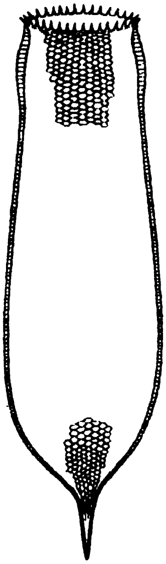
Lorica tall bell-shaped, 2.0–3.1 oral diameters in length; oral rim denticulate with about 48 triangular teeth; bowl dilated slightly at the



Text-fig. 16. *Cyttarocylis magna* BRANDT ×200.



Text-fig 17. *Parafavella faceta*, n. sp. ×400.



Text-fig. 18. *Parafavella ventricosa* (JÖRGENSEN) ×250.

anterior 0.2 of the total length, its widest transdiameter 1.07 oral diameters, below the suboral expansion contracting gradually, changing from 10°–15° at the middle to 70°–90° in the aboral region; aboral horn stout, shortened, conical (20°–32°), 0.07–0.09 of the total length in length, tip usually acute.

Length, 147(123–171)μ; oral diameter, 56(51–61)μ; greatest transdiameter, 60(54–65)μ.

Occurs in January; common.

Differs from *Parafavella obtusangula* (OSTENFELD) and *P. parumdentata* (BRANDT) in more abrupt contraction of the aboral region and in having a more distinct aboral horn and from *P. pacifica* HADA in having many teeth of the oral rim and in the slowly contracting aboral part.

24. **Parafavella ventricosa** (JÖRGENSEN) KOFOID and CAMPBELL.

Text-figure 18.

Cyttarocylis denticulata var. β *cylindrica forma ventricosa* JÖRGENSEN. 1899, p. 34, pl. 3, fig. 30.

Parafavella ventricosa, KOFOID and CAMPBELL, 1929, p. 171, fig. 314; KOFOID, 1930, fig. 30 (No. 314),

Lorica finger-shaped, 5 oral diameters in length; oral margin denticulated with comparatively fewer teeth; bowl subcylindrical, with a slight suboral bulge, enlarged gradually towards the aboral end and widest at the posterior 0.37 of the total length, its greatest transdiameter 1.25 oral diameters; aboral region an inverted convex cone of 78°; aboral horn 0.1 of the total length in length, conical (8°), tip more or less acute.

Length, 316 μ; oral diameter, 64 μ; greatest transdiameter, 80μ.

Occurs in August; very rare.

Differs from *Parafavella gigantea* (BRANDT) in the bulbous aboral region, in the shorter horn, and in fewer teeth on the oral margin.

25. **Parafavella subrotundata** (JÖRGENSEN) KOFOID and CAMPBELL.

Cyttarocylis denticulata var. γ *subrotundata* JÖRGENSEN, 1899, p. 34, pl. 2, figs. 20, 21.

Parafavella subrotundata. KOFOID and CAMPBELL, 1929, p. 170, fig. 316; HADA, 1932 b, p. 54, fig. 1.

Length, 220(211–228)μ; oral diameter, 62(58–63)μ.

Occurs in February and March; rare.

Family **Ptychocylidae.**

Genus PTYCHOCYLIS BRANDT, 1896.

26. **Ptychocylis obtusa** BRANDT.

Ptychocylis obtusa BRANDT, 1896, p, 59, pl. 3, fig. 15; KOFOID and CAMPBELL. 1929, p. 188, fig. 349; HADA, 1932 b, p. 55, fig. 21.

Length, 98(97–112)μ; oral diameter, 63(60–71)μ.
Occurs in January-July; common.

27. **Ptychocylis drygalskii** BRANDT.

Text-figure 19.

Ptychocylis Drygalskii BRANDT, 1896, p. 59, pl. 3, fig. 14; KOFOID and CAMPBELL, 1929, p. 188, fig. 350.
Ptychocylis urnula var. *digitalis* JÖRGENSEN, 1901, p. 17, pl. 2, figs. 29, 30.
Ptychocylis urnula var. *digitalis* forma *subintegerrima* JÖRGENSEN, 1901, p. 26, pl. 3, fig. 31.
Ptychocylis obtusa var. *drygalskyi* (part), BRANDT. 1906, pl. 55, figs. 1 3, pl. 56, figs. 3, 3 a, pl. 57, fig 10; 1907, p. 312.
Ptychocylis ventricosa MEUNIER, 1910, p. 127, pl. 10, fig. 3.

Lorica wide goblet-shaped, 1.4 oral diameters in length; oral rim regularly denticulated; bowl generally a low, inverted, convex cone with two distinct expansions: each respectively 1.15 and 1.20 oral diameters in transdiameter; aboral region concave conical 90°; aboral end broadly rounded, more or less thicker than the other parts, with a rugose surface.

Length, 77μ; oral diameter, 55μ.

Occurs in July; very rare.

Differs from *Ptychocylis obtusa* BRANDT in stouter proportions and in the shape of the aboral cone.

Family **Petalotrichidae.**

Genus ACANTHOSTOMELLA JÖRGENSEN, 1927.

28. **Acanthostomella norvegica** (DADAY) JÖRGENSEN.

Text-figure 20.

Amphorella norvegica DADAY, 1887, p. 543.
Acanthostomella norvegica, KOFOID and CAMPBELL, 1929, p. 193, fig. 363; HADA, 1932 b, p. 56, fig. 22.

Length, 43–47μ; oral diameter, 29–30μ; greatest transdiameter, 31–32μ.

Occurs in February and March; rare.

Family Xystonellidae.

Genus PARUNDELLA JÖRGENSEN, 1924.

29. Parundella pellucida (JÖRGENSEN) KOFOID and CAMPBELL.

Text-figure 21.

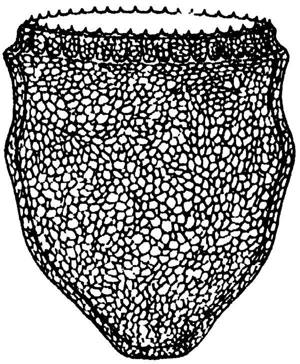
Undella pellucida (part) JÖRGENSEN, 1899, p. 41, pl. 1, fig. 7.
Parundella pellucida, KOFOID and CAMPBELL, 1929, p. 233, fig. 438.

Lorica elongate chalice-shaped, 4 oral diameters in length; oral rim entire; bowl subcylindrical, slightly contracting in the anterior 0.25 of the total length; aboral region tapering (45°) to a caudal spine; lance conical (18°), 0.25 of the total length in length, single-lamellate in the posterior 0.42 of its length, with three somewhat spiral costae on the upper thick-walled part, tip pointed.

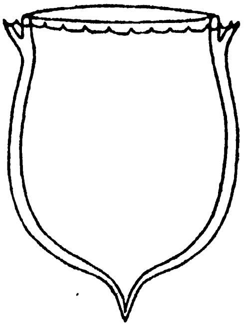
Length, 102 μ; oral diameter, 27 μ.

Occurs in November; very rare.

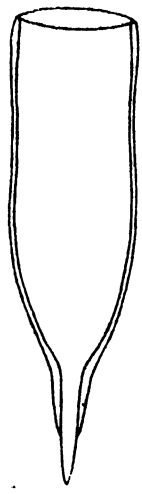
Differs from *Parundella caudata* (OSTENFELD) in the absence of predominant fins at the junction of the aboral region and the lance.



Text-fig. 19. *Ptychocylis drygalskii* BRANDT ×600.



Text-fig. 20. *Acanthostomella norvegica* (DADAY) ×1000.



Text-fig. 21. *Parundella pellucida* (JÖRGENSEN) ×600.

Family Undellidae.

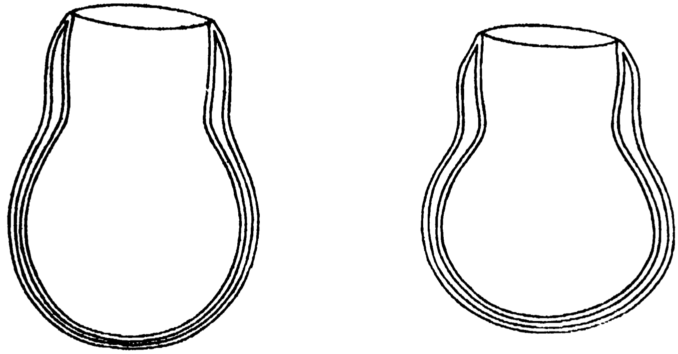
Genus PROPLECTELLA KOFOID and CAMPBELL, 1929.

30. Proplectella expolita, n. sp.

Text-figure 22.

Lorica stout flask-shaped, with a well-developed outer collar, 2.00–2.58

oral diameters in length; suboral region subcylindrical, thinning outwardly to a sharp oral rim, its length 0.30–0.33 of the total length; bowl generally subspherical, sometimes broadly ovate, 1.5–2.0 oral diameters



Text-fig. 22. *Proplectella expolita*, n. sp ×750.

in transdiameter; aboral end usually hemisherical or widely rounded, occasionally showing weakly a trace of bluntly pointing; wall thickened at the basal part of the collar, thinning gradually towards the aboral end, about 0.15 of the oral diameter in thickness in the thickest wall of the collar.

Length, 66(56–76)μ; oral diameter, 27(24 30)μ; transdiameter of the bowl, 49(44–52)μ.

Occurs in June-August; common.

Differs from all other species of *Proplectella* in having the conspicuous collar and from *Undella californiensis* KOFOID and CAMPBELL in the structure of the wall which is uniform in thickness in that species, but in this species is thickest at the lower part of the collar and thinnest at the aboral end.

Family Tintinnidae.

Genus AMPHORELLA DADAY, 1887.

31. Amphorella brandti JÖRGENSEN.

Text-figure 23.

Tintinnus amphora, BRANDT, 1906, pl. 69, fig. 6; 1907 (part), p. 433.
Amphorella quadrilineata var. *brandti* JORGENSEN, * 1924.
Amphorella brandti, KOFOID and CAMPBELL, 1929, p. 309, fig. 588.

Lorica 2.2–2.6 oral diameters in length, consisting of a collar of the circular cross-section and a triangular bowl, narrowest at the base of the

collar, its smallest transdiameter 0.71–1.79 of the oral diameter; collar an inverted, truncated, concave cone of 65°–75°; bowl cylindrical in the little upper part, triangular in the lower region with three prismatic longitudinal ridges; aboral end transversely concave; wall having separated lamellae in the anterior region of the lorica, fused posteriorly into a single lamina and thinning.

Length, 119(100–128)μ; oral diameter, 44(42–46)μ.

Occurs in March-October; common.

Differs from *Amphorella quadrilineata* (CLAPARÈDE and LACHMANN) in having three instead of four fins.

Genus TINTINNUS SCHRANK, 1803.

32. Tintinnus exigua, n. sp.

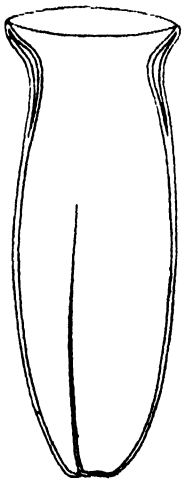
Text-figure 24.

Lorica an elongate truncated cone of 3°–5°, 3.5–4.2 oral diameters in length; oral end abruptly flaring to form a marked brim; sides nearly straight; aboral end 0.64–0.75 of the oral diameter in aboral diameter, without a brim and a flare.

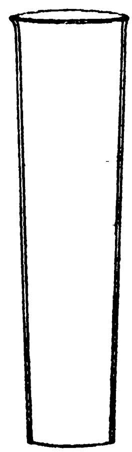
Length, 148(130–160)μ; oral diameter, 38(36–40)μ.

Occurs in May-October; common.

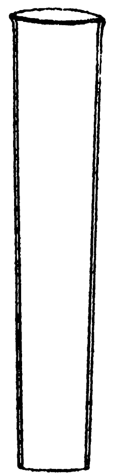
Differs from *Tintinnus tenuis* KOFOID and CAMPBELL in being smaller and more widely conical.



Text-fig. 23. *Amphorella brandti* JÖRGENSEN ×500.



Text-fig. 24. *Tintinnus exigua*, n. sp. ×400.



Text-fig. 25. *Titinnus tenuis* KOFOID and CAMPBELL ×250.

33. Tintinnus tenuis KOFOID and CAMPBELL.

Text-figure 25.

Tintinnus lusus-undae (part), ZACHARIAS, 1906, p 518, fig. 6; BRANDT, 1907, p. 420.
Tintinnus lusus-undae var. c (part), BRANDT, 1906, pl. 65, fig. 19; 1907, p. 422.
Tintinnus tenue KOFOID and CAMPBELL, 1929, p. 339, fig. 655.

Lorica a long, inverted, truncated cone of 2°–3°, with very slightly expansion in the middle part, its length 4.4-5.9 oral diameters; both ends entire, oral one flaring with a brim, the other without a flare, 0.64–0.77 of the oral diameter in aboral diameter.

Length, 261(236-312)μ; oral diameter, 51(49–56)μ.

Occurs in June-September; common.

Differs from *Tintinnus fraknóii* DADAY in the absence of the aboral flare and from *T. lusus-undae* ENTZ in more slender proportions.

Genus SALPINGELLA JÖRGENSEN, 1924.

34. Salpingella attenuata JÖRGENSEN.

Text-figure 26.

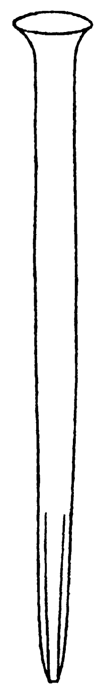
Tintinnus acuminatus, ENTZ, Sr., 1885, p. 201, pl. 14, fig. 13.
Tintinnus acuminatus var. c *glockentogeri* (part) BRANDT, 1906, pl. 68, fig. 2-4; 1907, p. 390.
Salpingella acuminata subsp. *glockentögeri* var. *attenuata* JÖRGENSEN, *1924.
Salpingella attenuata, KOFOID and CAMPBELL, 1929, p. 351, fig. 687.

Lorica much elongated, slender, 9 oral diameters in length; consisting of a funnel-shaped collar and a long tubular bowl; collar a wide inverted concave cone of 60°; bowl cylindrical in the posterior half, 0.5 of the oral diameter in length, sharply conical (5°) in the posterior half, distally convex conical (30°); aboral end open, truncate; aboral region with 6 somewhat dexiotropic fins on the posterior 0.29 of the total length.

Length, 288 μ; oral diameter, 32 μ; transdiameter of the bowl, 16 μ.

Occurs in November; very rare.

Differs from *Salpingella gracilis* KOFOID and CAMPBELL in size and in having fewer fins and



Text-fig. 26. *Salpingella attenuata* JÖRGENSEN $\times 300$

from *S. ricta* KOFOID and CAMPBELL in more slender proportions and in lack of the surface rugose.

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